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Can We Use Simple Models to Understand Complex Phenomena in Cytoskeleton Proteins?

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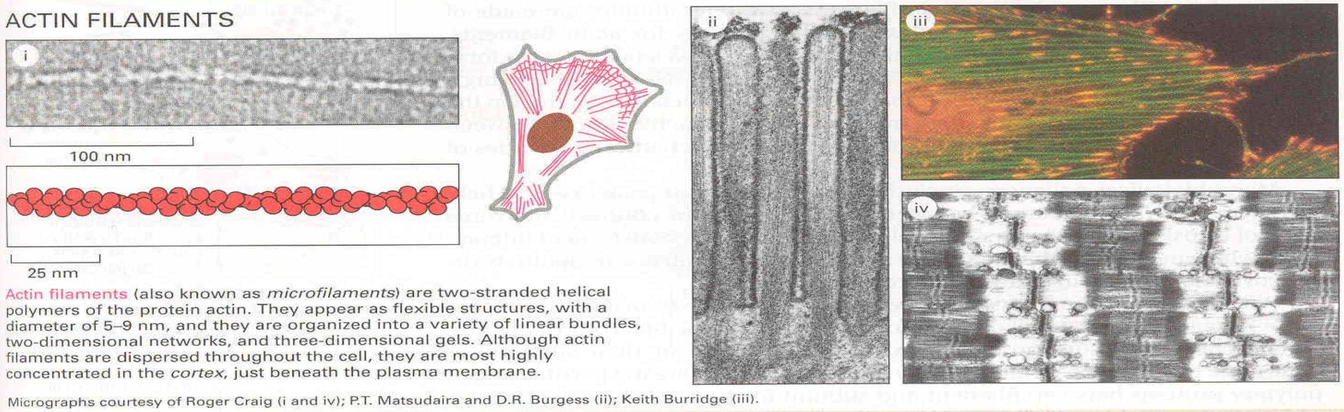
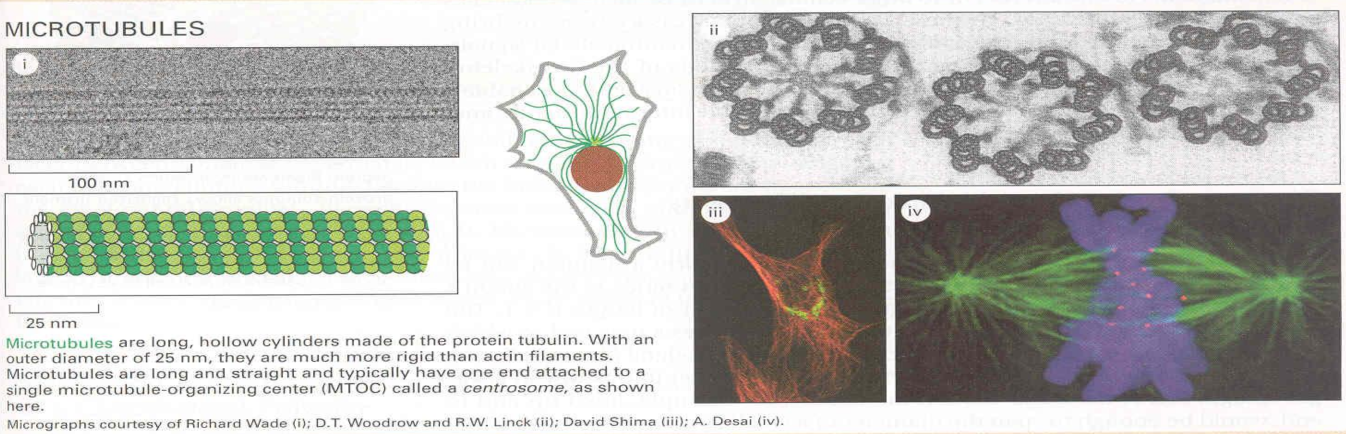
CYTOSKELETON PROTEINS



Actin filaments (also known as *microfilaments*) are two-stranded helical polymers of the protein actin. They appear as flexible structures, with a diameter of 5–9 nm, and they are organized into a variety of linear bundles, two-dimensional networks, and three-dimensional gels. Although actin filaments are dispersed throughout the cell, they are most highly concentrated in the *cortex*, just beneath the plasma membrane.

Micrographs courtesy of Roger Craig (i and iv); P.T. Matsudaira and D.R. Burgess (ii); Keith Burridge (iii).

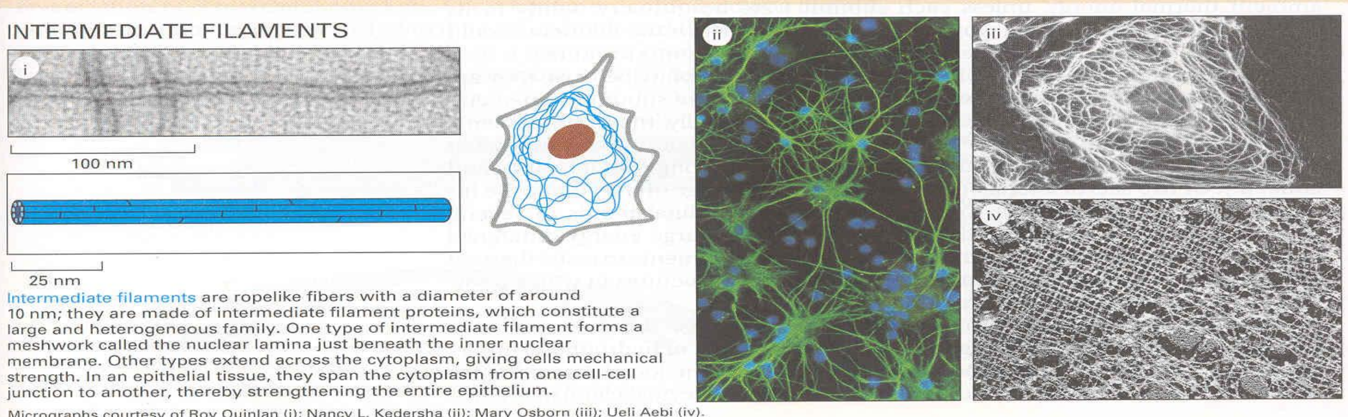
actin filaments



Microtubules are long, hollow cylinders made of the protein tubulin. With an outer diameter of 25 nm, they are much more rigid than actin filaments. Microtubules are long and straight and typically have one end attached to a single microtubule-organizing center (MTOC) called a *centrosome*, as shown here.

Micrographs courtesy of Richard Wade (i); D.T. Woodrow and R.W. Linck (ii); David Shima (iii); A. Desai (iv).

microtubules

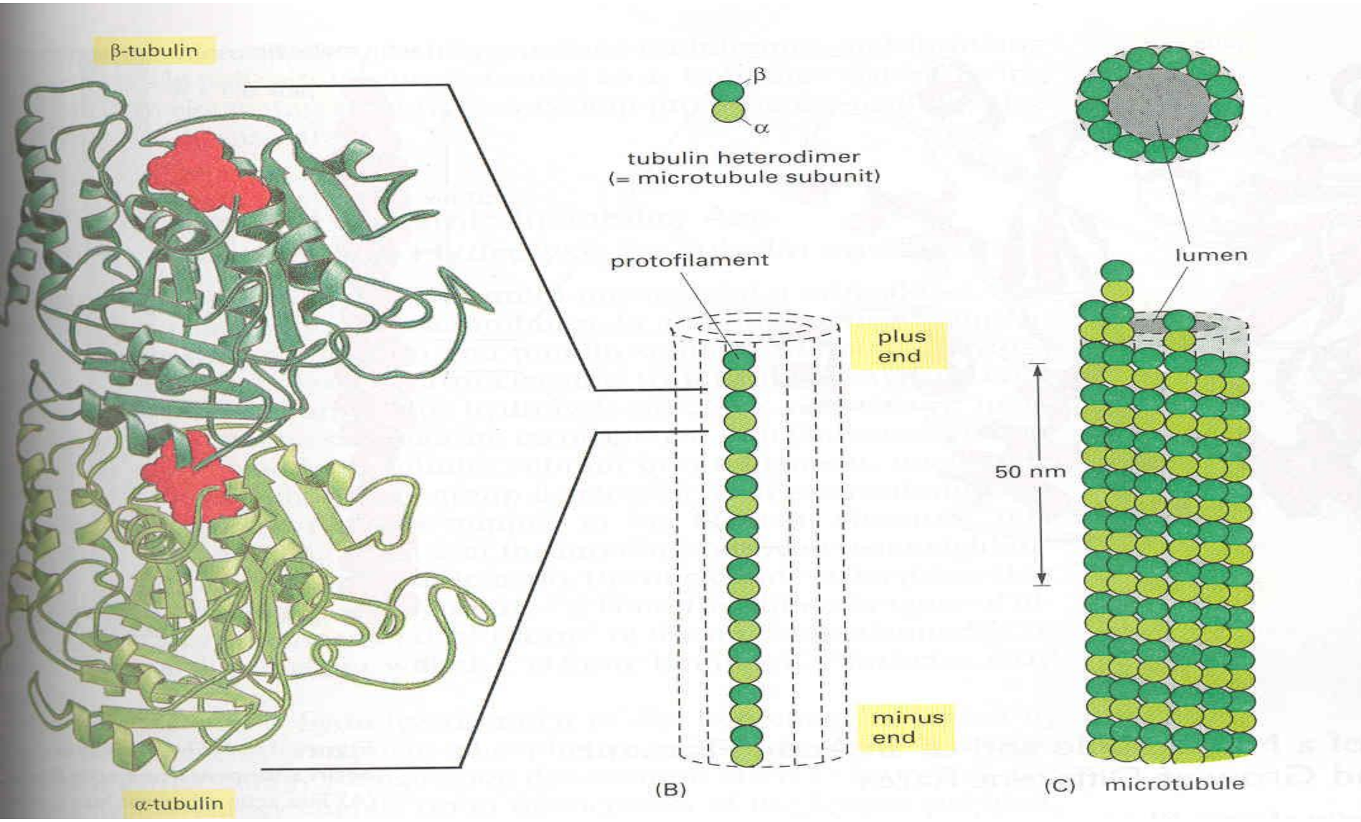


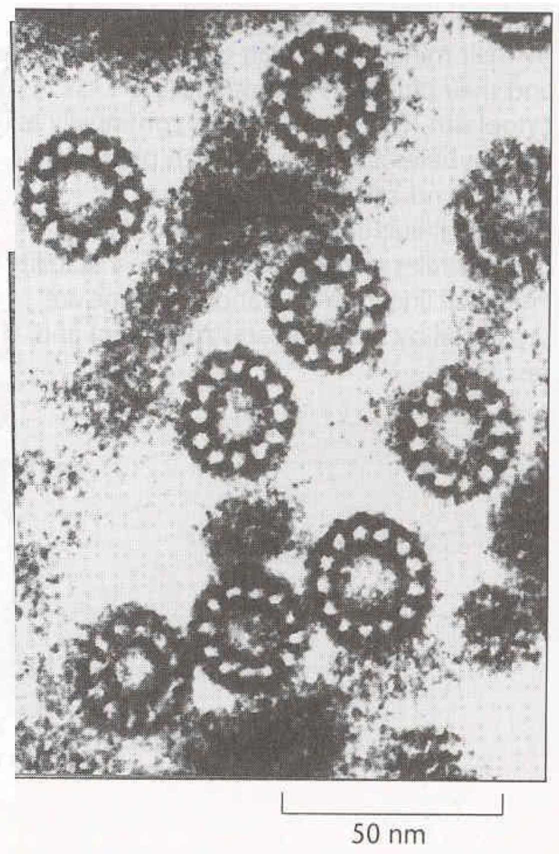
Intermediate filaments are ropelike fibers with a diameter of around 10 nm; they are made of intermediate filament proteins, which constitute a large and heterogeneous family. One type of intermediate filament forms a meshwork called the nuclear lamina just beneath the inner nuclear membrane. Other types extend across the cytoplasm, giving cells mechanical strength. In an epithelial tissue, they span the cytoplasm from one cell-cell junction to another, thereby strengthening the entire epithelium.

Micrographs courtesy of Roy Quinlan (i); Nancy L. Kedersha (ii); Mary Osborn (iii); Ueli Aebi (iv).

intermediate filaments

Microtubules



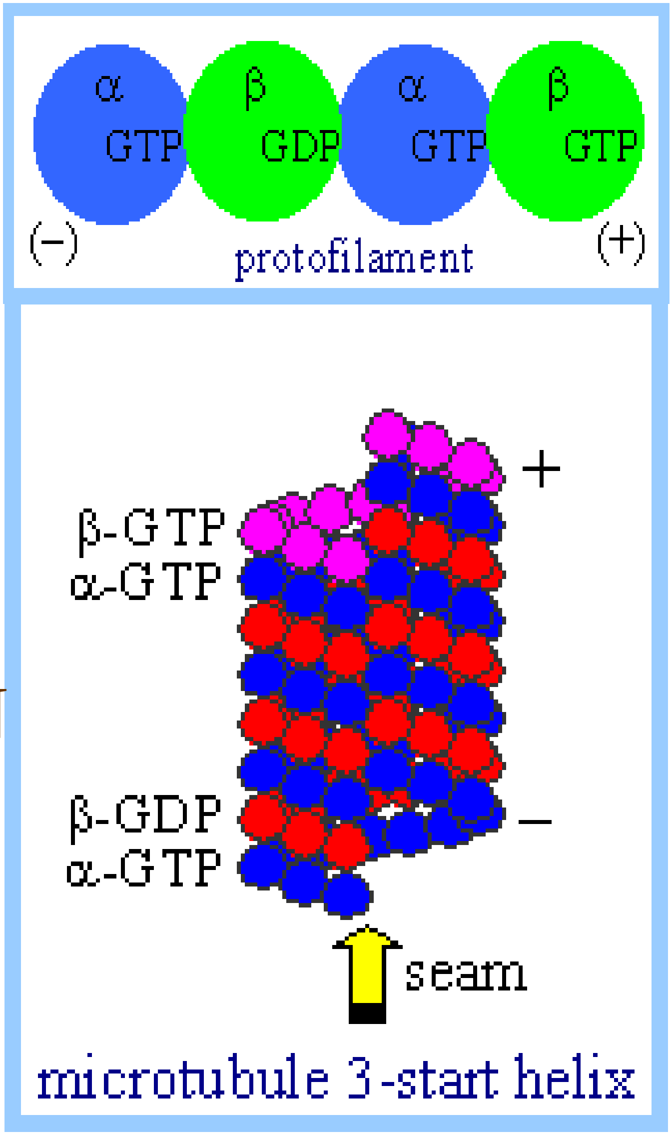


- **Rigid hollow cylindrical biopolymers**
- **Length-1-10 μm, diameter -25 nm, thickness of walls 5-6nm**
- **Number of protofilaments – 10-15, the most probable 13**

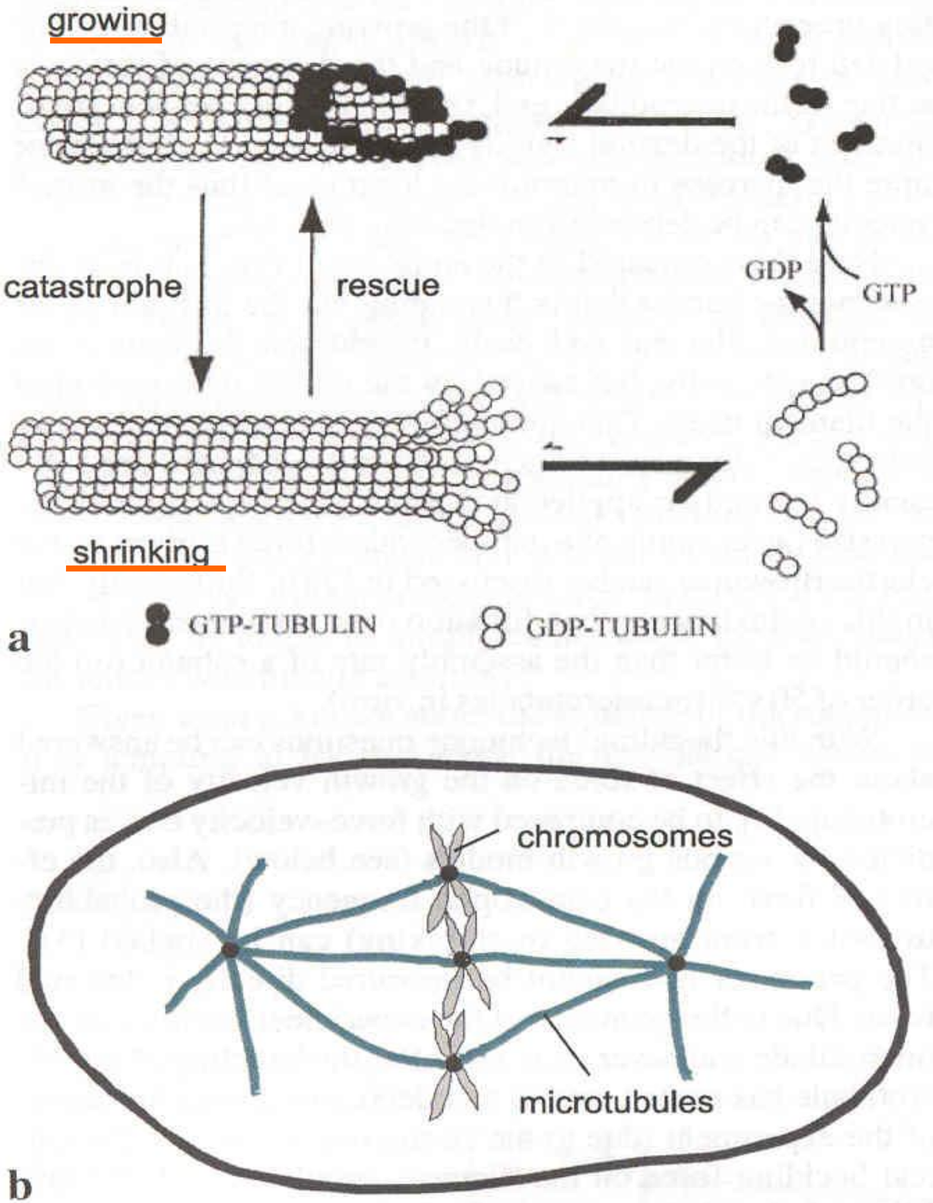
Microtubules

- 3-start helical structure with a seam
- αβ-tubulin-GTP subunit
- Size of the dimer subunit 8x4x4 nm
- Polar structure
- Plus ends grow faster than minus ends
- Polymerization produces forces 1-20pN
- Rigid biopolymers: persistence length

5 mm(!!!)



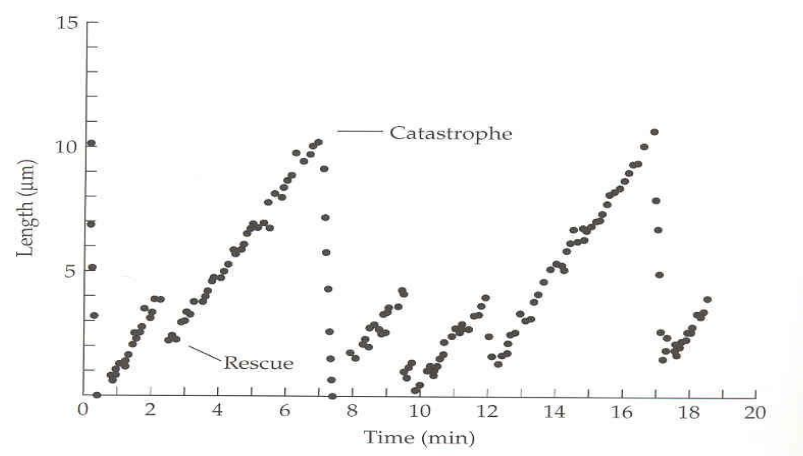
Microtubules: Dynamic Instability



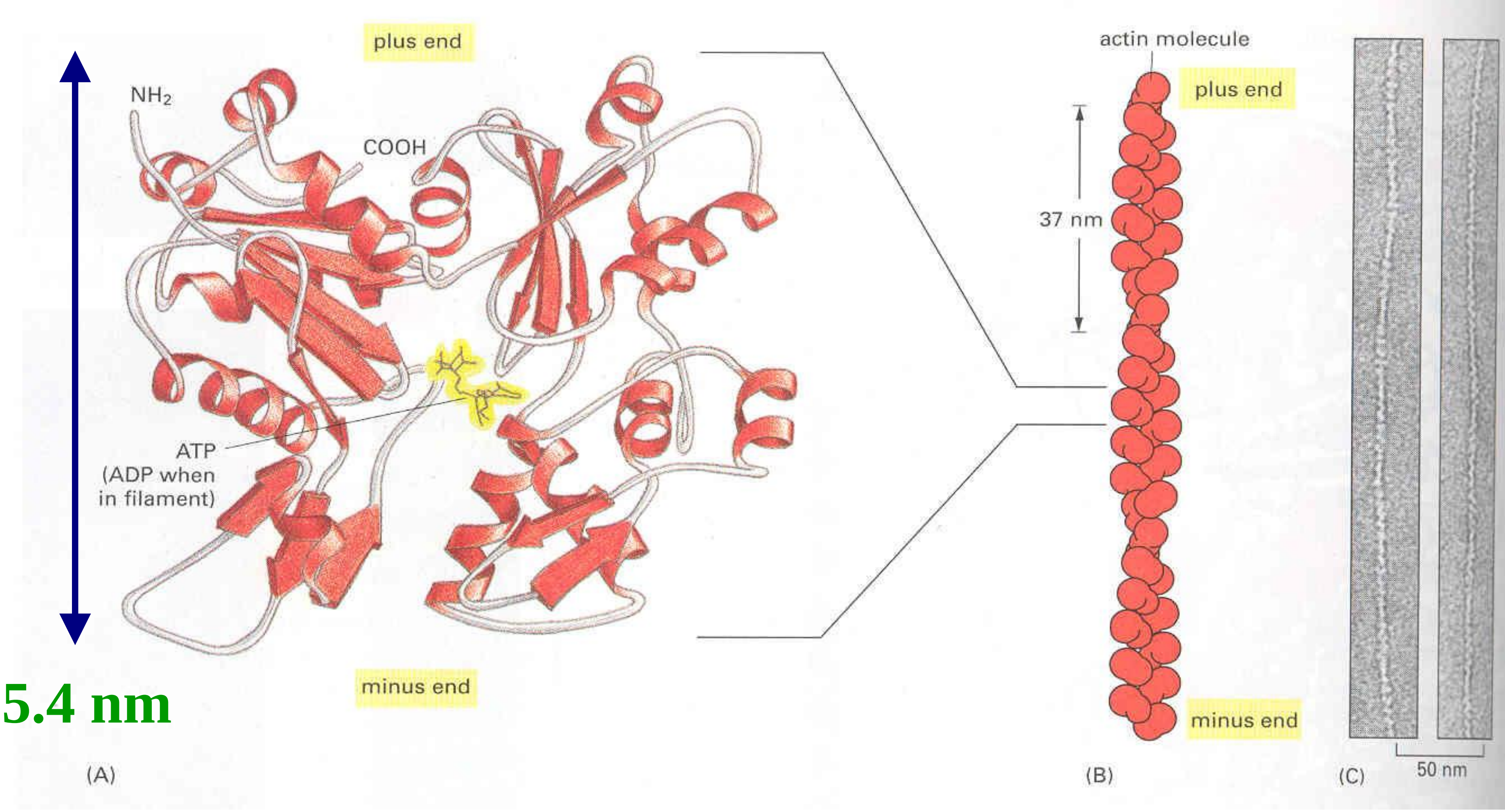
Microtubules exist in two dynamic phases: **growing or shrinking**

Dynamic instability – non-equilibrium phenomenon

Mechanisms of dynamic instability are not understood

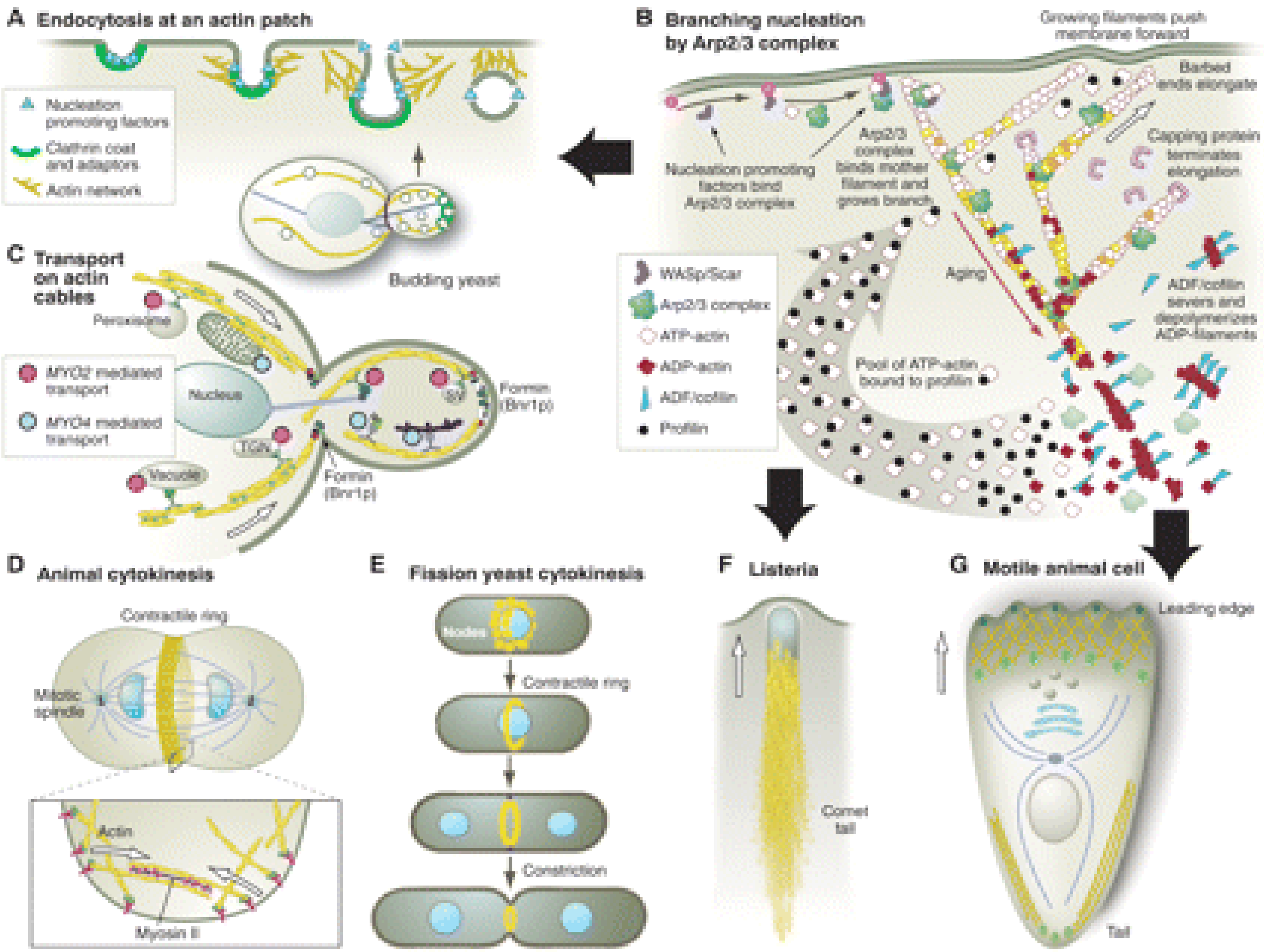


Actin Filaments:



Two-stranded right handed helix polymer. Protofilaments are half-staggered and wrapped around each other with a 74 nm period; ATP molecules hydrolyze inside

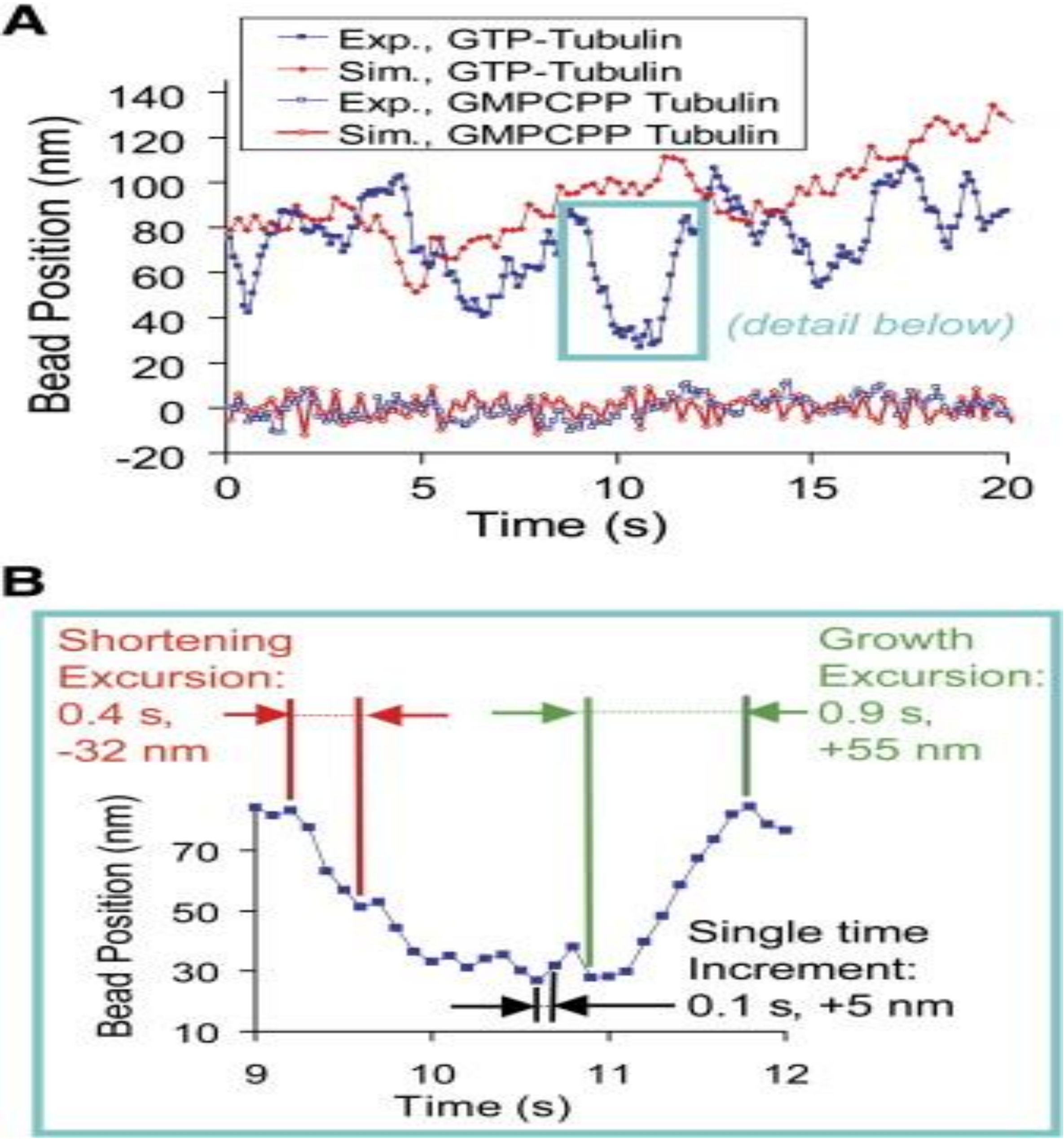
Biological Functions



Cytoskeleton proteins are critically important for cellular transport, motility and cell division

Science, **326**, 1208 (2009)

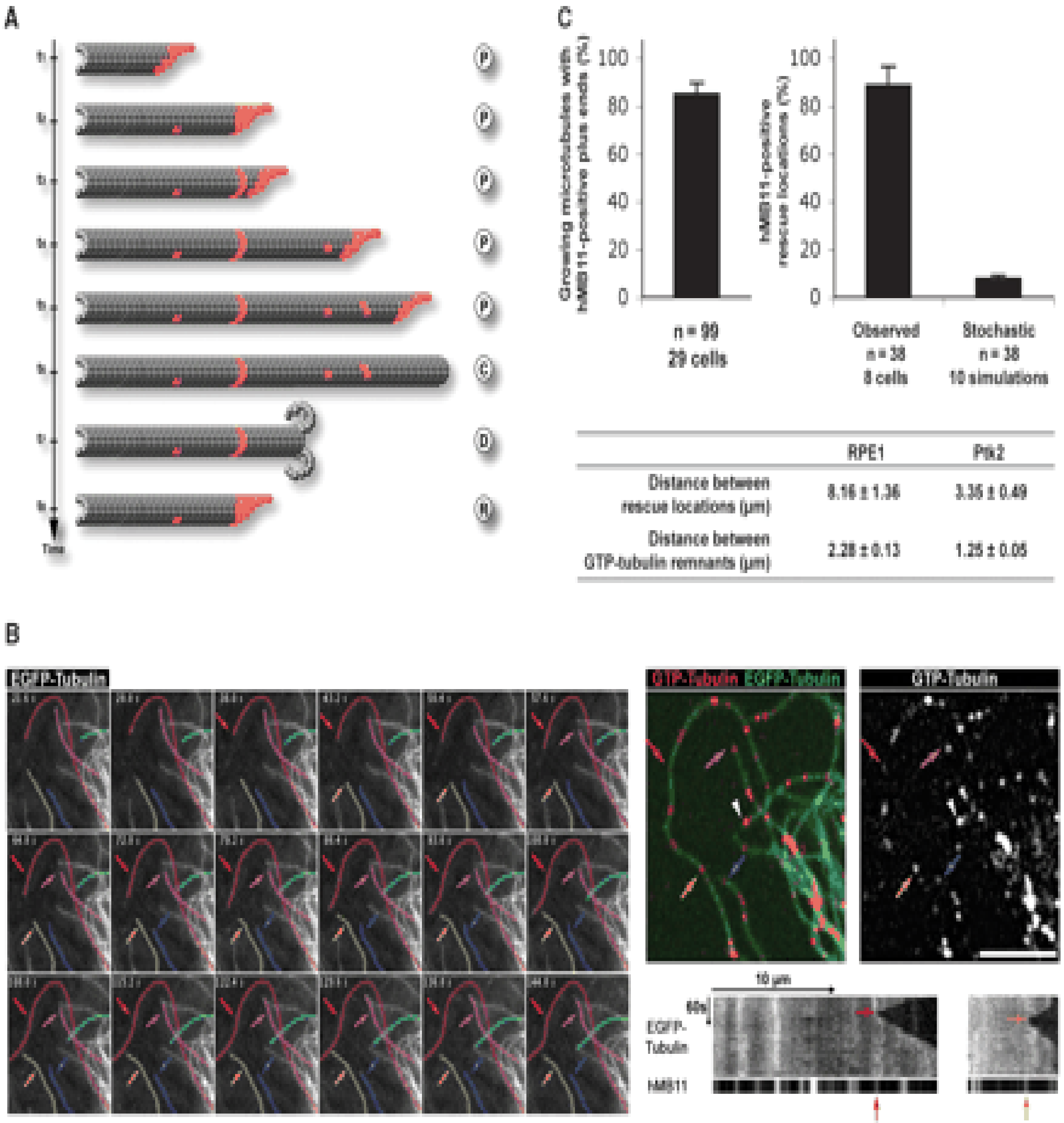
High-Resolution Experiments



Observation of microtubule growth with nanoscale resolution:

Curr. Biol. **17**, 1445 (2007)

Experiments

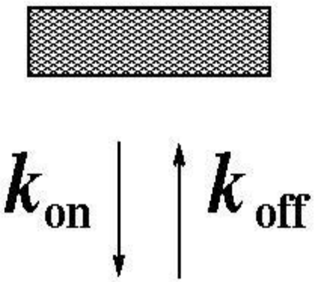


Observation of GTP-tubulin monomers in vivo and microtbule rescues

Science **322**, 1353 (2008)

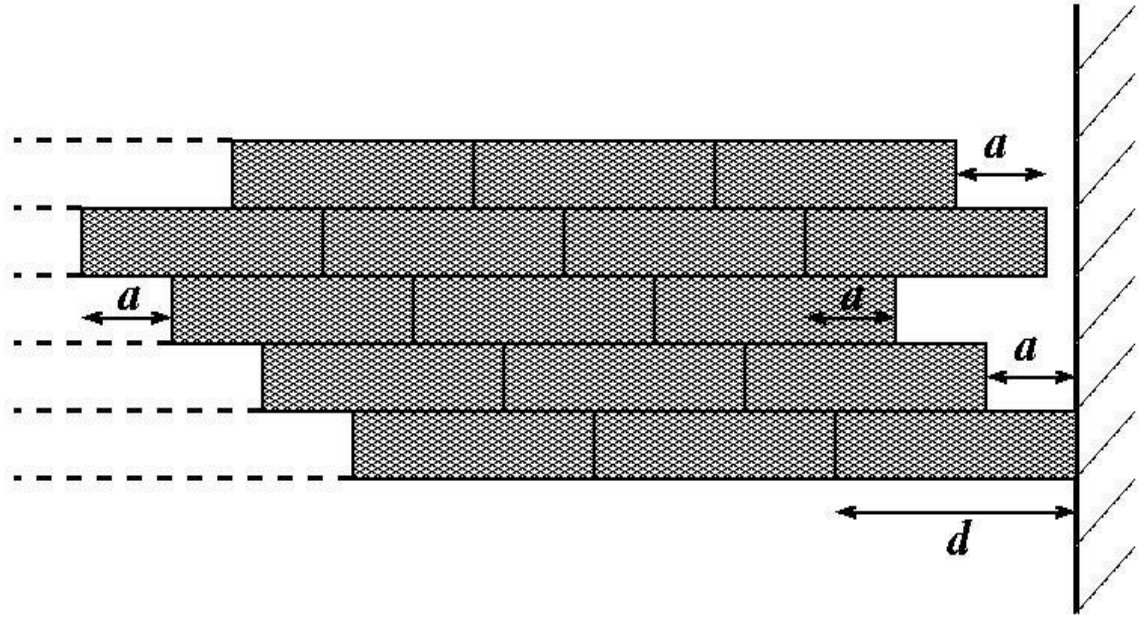
Theoretical Modeling. Multi-Scale Approach:

1) Macroscopic - phenomenological models



Balance between polymerization and depolymerization processes

$$V = k_{on}c - k_{off}$$



Structure of the biopolymers, internal interactions, different biochemical transitions and states are neglected

Microtubules: Phenomenological Model

Phenomenological ("Thermodynamic") Theory: Dogterom and Yurke (*Science*,1997)

$$V(F) = d_0[k_{on}\exp(-\theta^+ F d_1 / k_B T) - k_{off}]$$

Assumption: $d_0 = d_1 = d/13 = 0.63$ nm

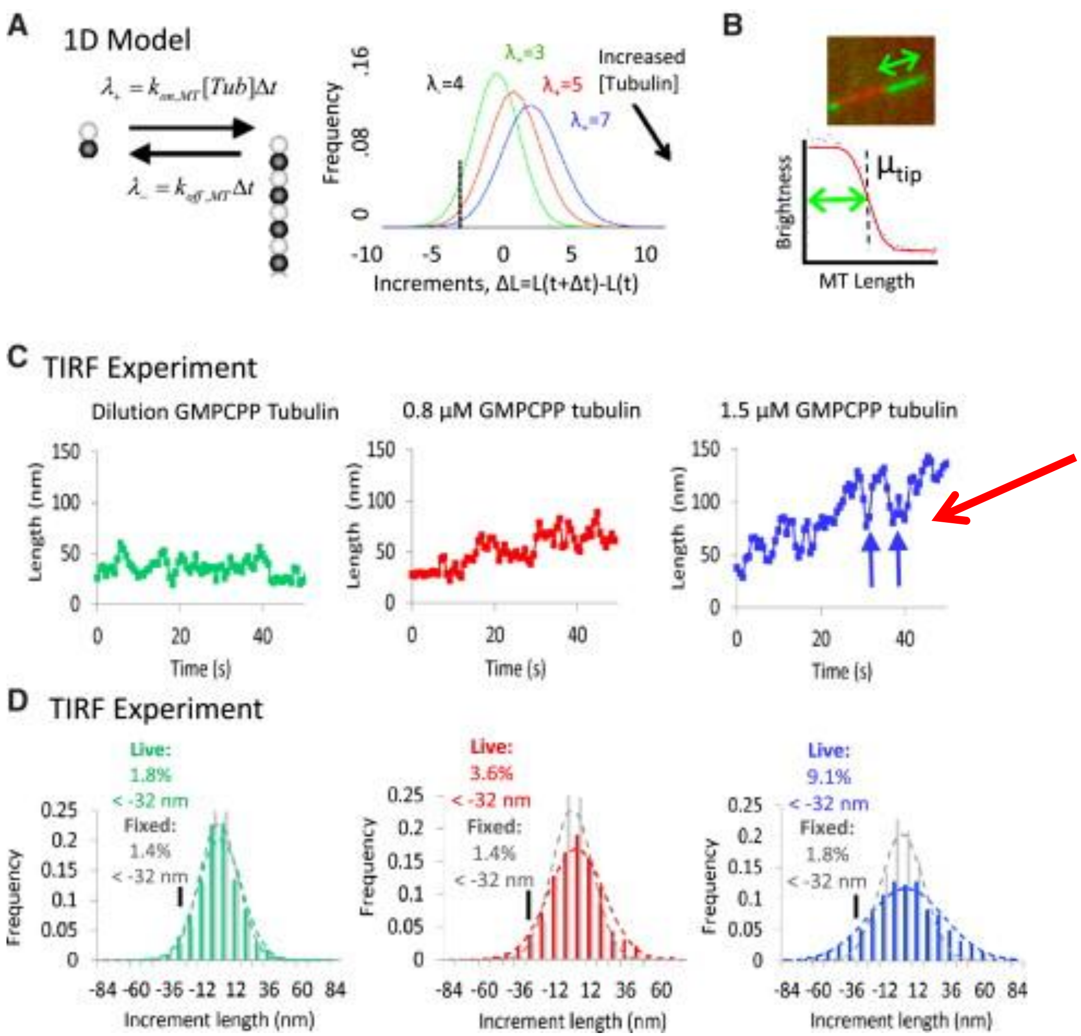
Fit of experimental data → **$k_{on} = 1791$ min^{-1}**

$k_{off} = -127$ min^{-1}

Unphysical! Chemical rates are always >0!

Phenomenological theory wrong!

Microtubules: Phenomenological Model



Direct observation of microtubule assembly via TIRF reveals that the tubulin off rates increase at higher free-tubulin concentration!!!

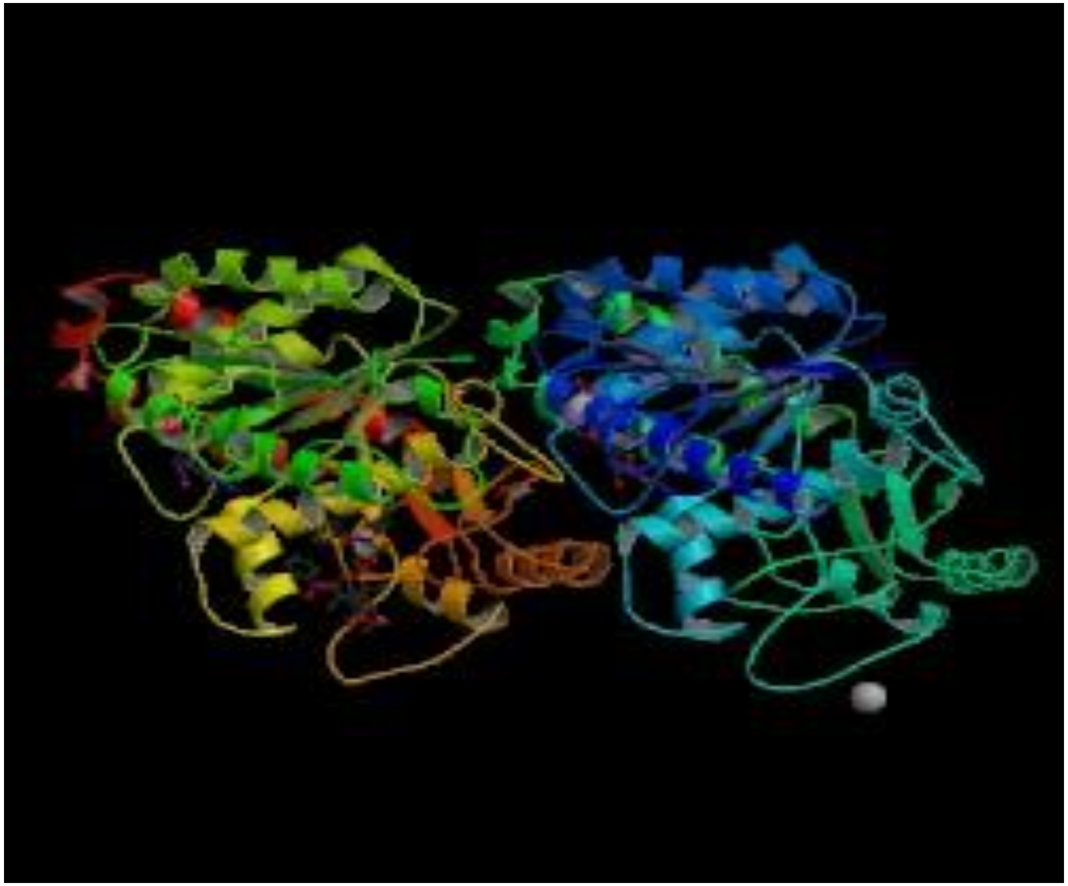
$$V = k_{on}c - k_{off}$$

Cell, **146**, 582 (2011)

Phenomenological theory wrong!

Theoretical Modeling. Multi-Scale Approach:

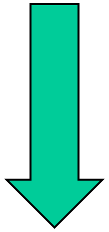
2) Microscopic approach – full atomistic simulations. Currently – do not exist for filaments!



Protein Data Bank:

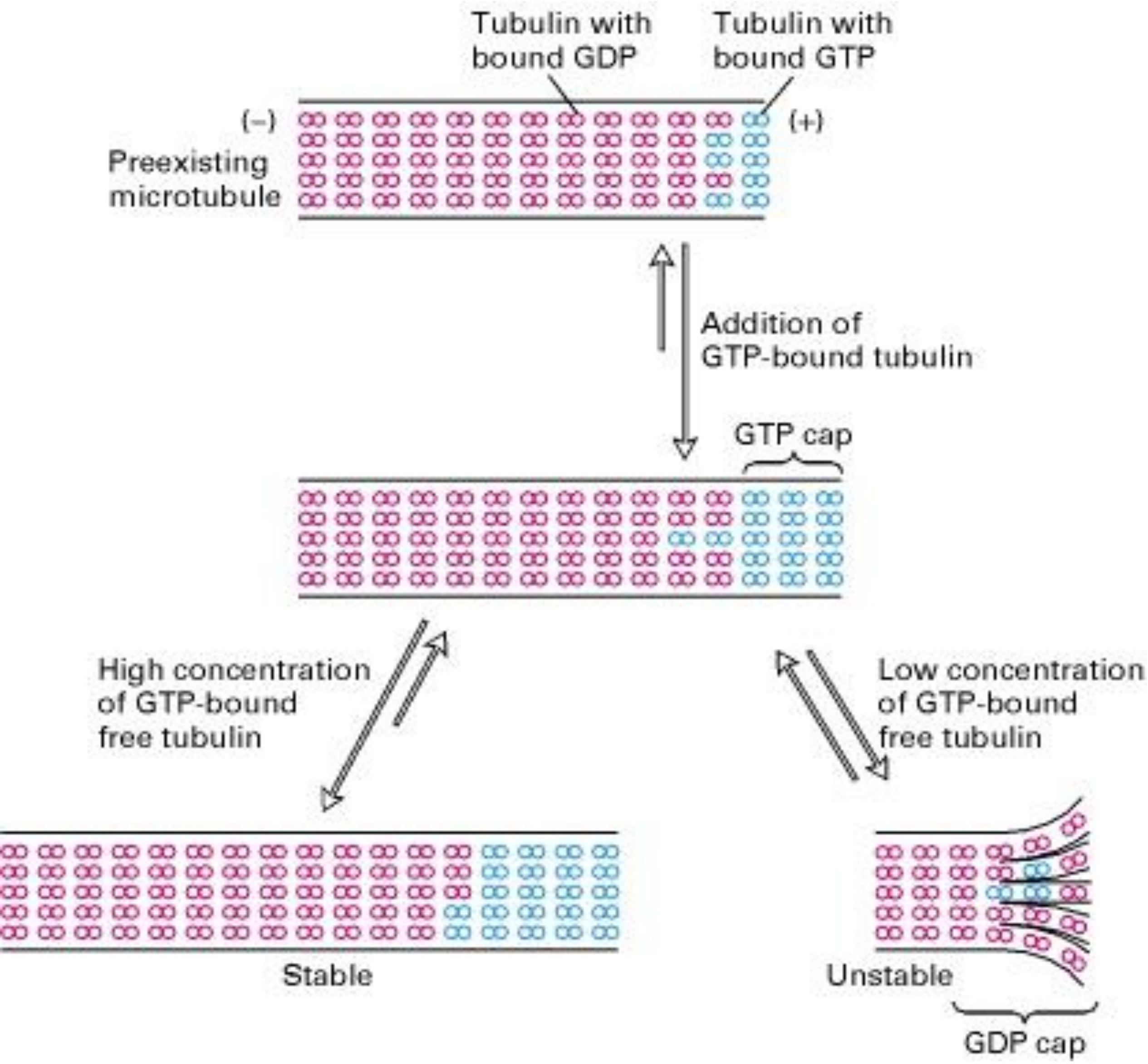
α-β tubulin subunit

More than 10000 atoms!!!



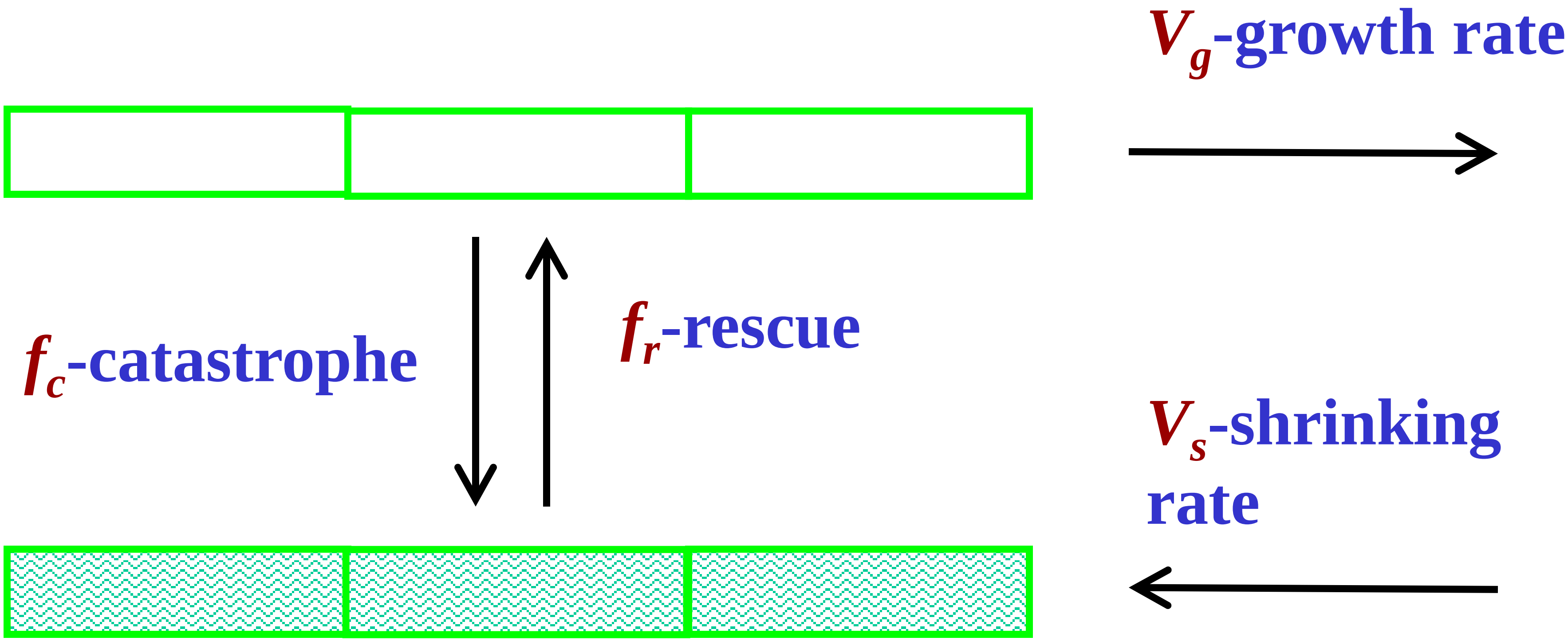
3) Mesoscopic approach

Current View on Dynamic Instability



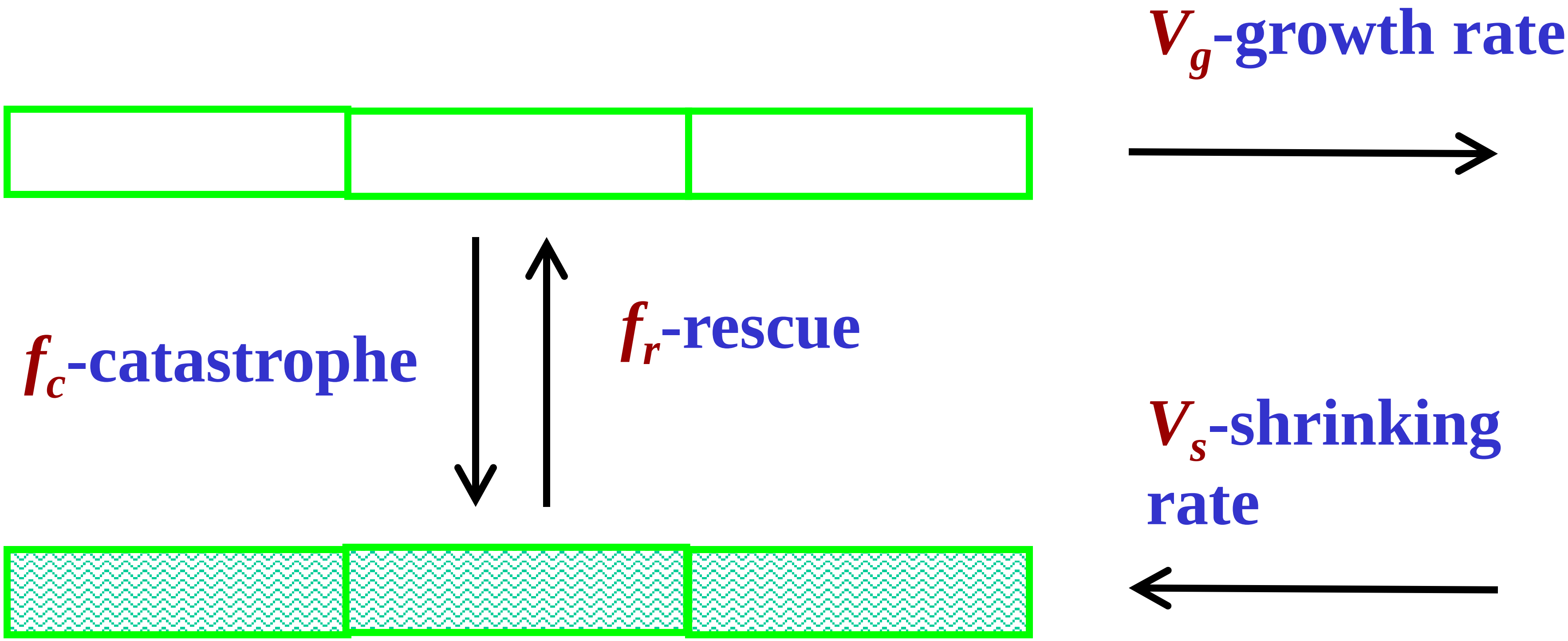
It is assumed that there is a cap of unhydrolyzed tubulin subunits at the end of microtubules. When the cap is removed – there is a catastophe event

Current View on Dynamic Instability



2-state phenomenological models: *Phys. Rev. Lett*. **70**, 1347 (1993); *PNAS USA* **81**, 6728 (1984); *Phys. Rev. E* **54**, 5538 (1996);…

Current View on Dynamic Instability

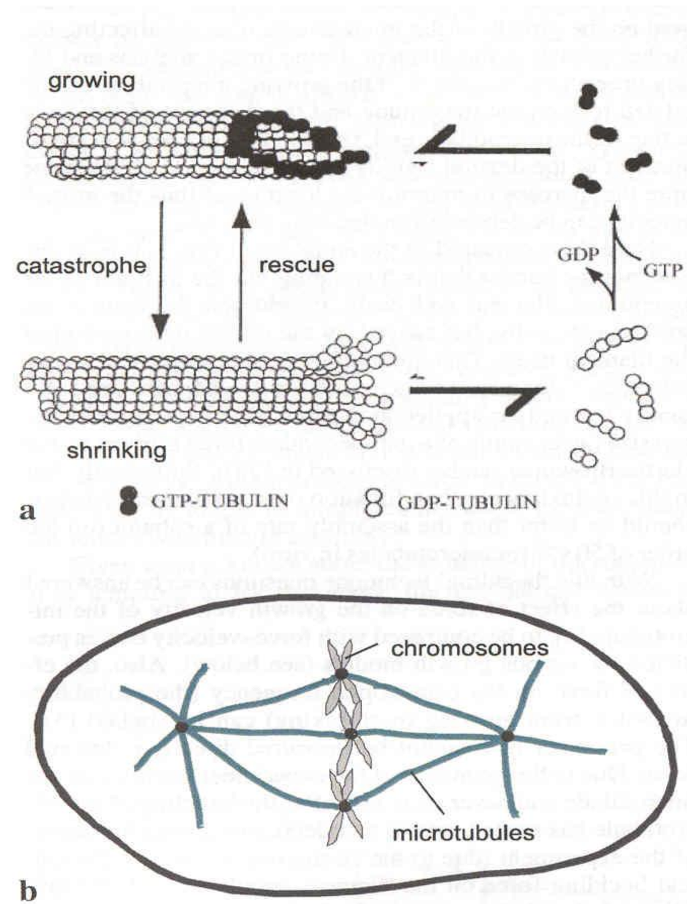


Problems: 1) cannot explain mechanisms of dynamic instability; 2) inconsistent with thermodynamics; 3) cannot explain single-molecule experiments; …

Previous Theoretical Efforts

Significant theoretical advances in the field from different groups:

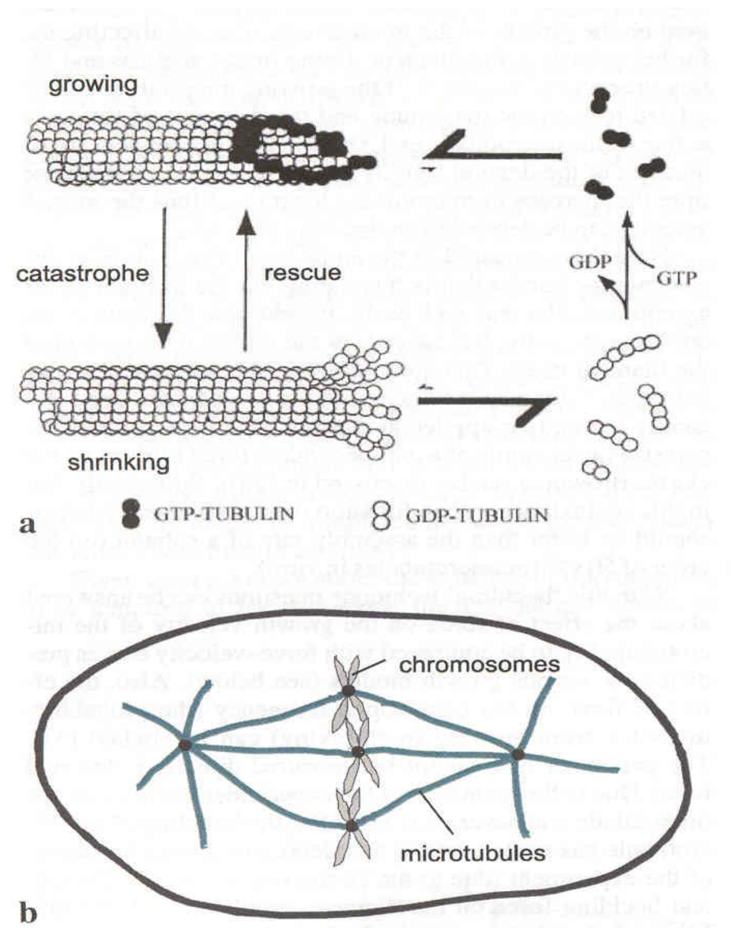
S. Redner, P. Krapivsky, T. Antal, J.-F. Joanny, F. Nedelec, B. Chakraborty, R. Lipowsky, J. Kierfield, M. Alber, H. Flyvbjerg, D. Odde, …



Our Theoretical Approach

Our Idea:

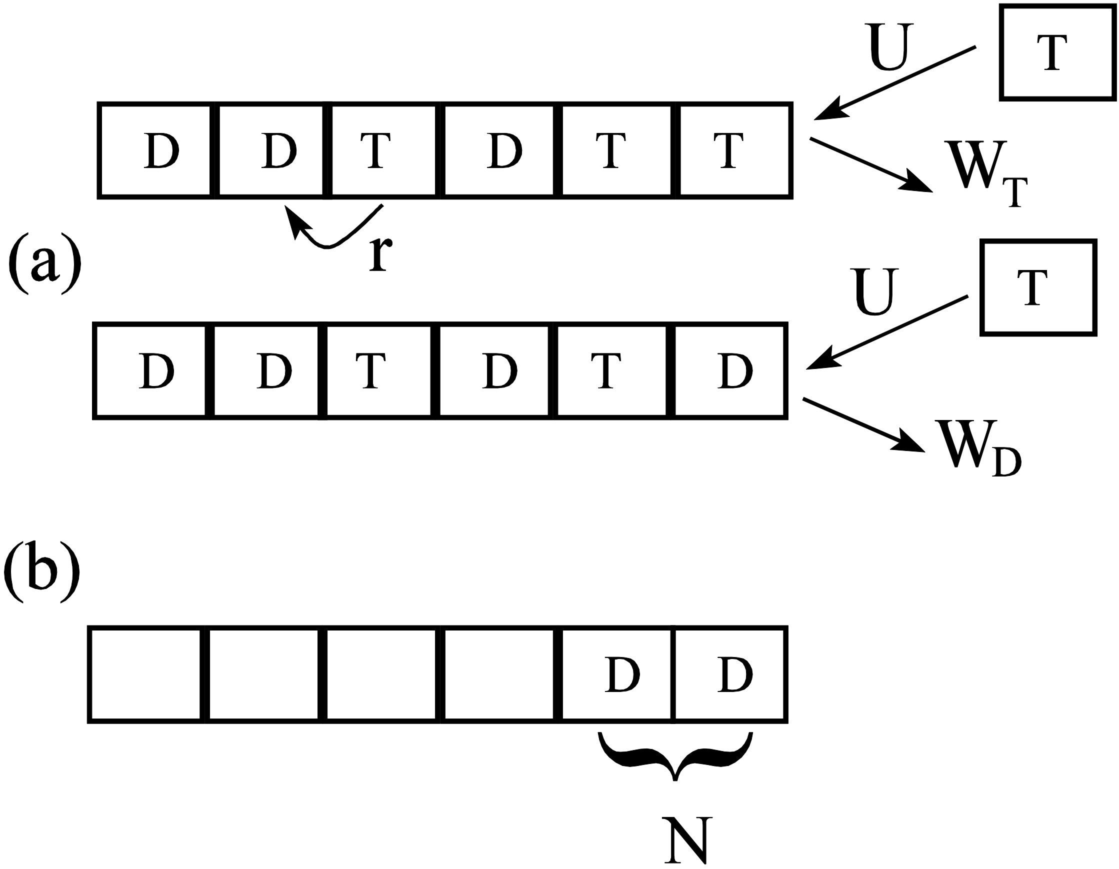
1) **Mechanisms of dynamic instability in microtubules are determined by intrinsic biochemical processes**



2) **We would like to develop a minimalist dynamic model that will take into account relevant biochemical transitions and discreteness of the system, and will also explain experimental trends**

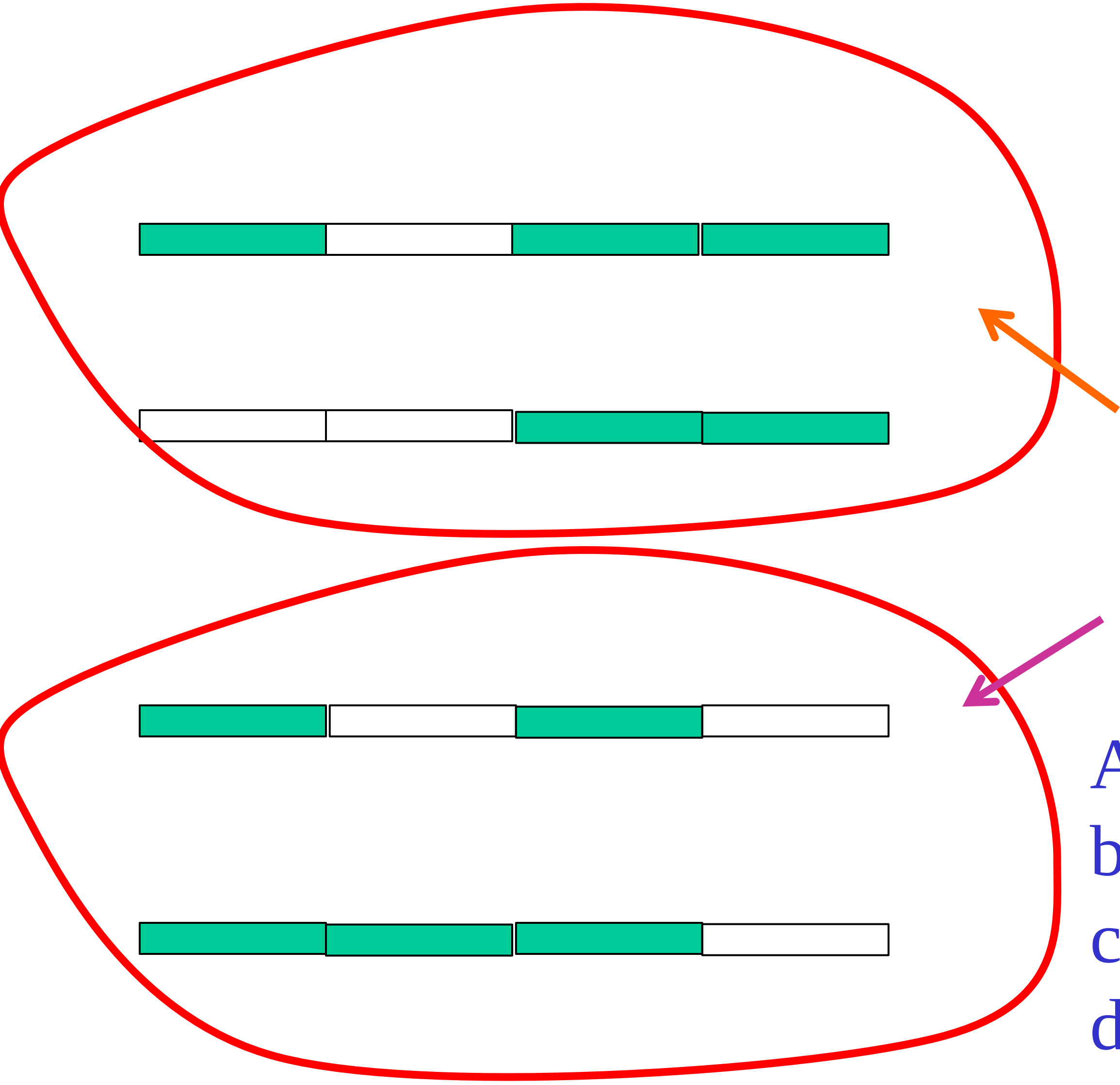
Our Model

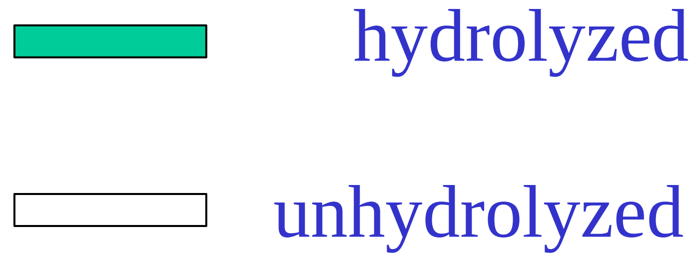
1) Discrete-state 1D non-equilibrium model that takes into account association/dissociation of tubulins and hydrolysis
2) random hydrolysis is assumed
3) Protofilament structure of microtubules is neglected



The model can be solved analytically in the mean-field approximation

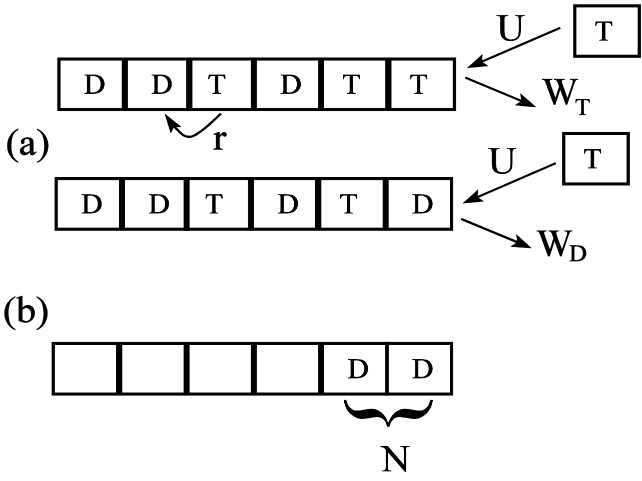
Our Model:2 Dynamic Phases





Any chemical transition between polymer configurations from2 different groups is associated with catastrophes or rescues

Our Model



We define catastrophes and rescues from nucleotide content of terminal regions.

Shrinking phase: all configurations that have last ***N*** units hydrolyzed

Growing phase: all other configurations

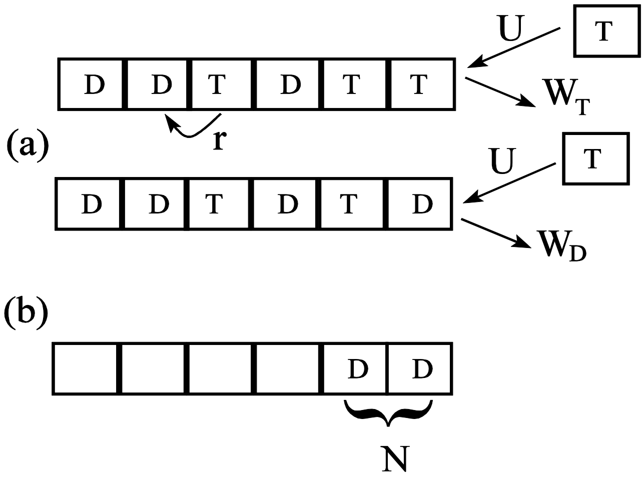
$f_c(N)$- catastrophe frequency = probability flux out of growing phase;

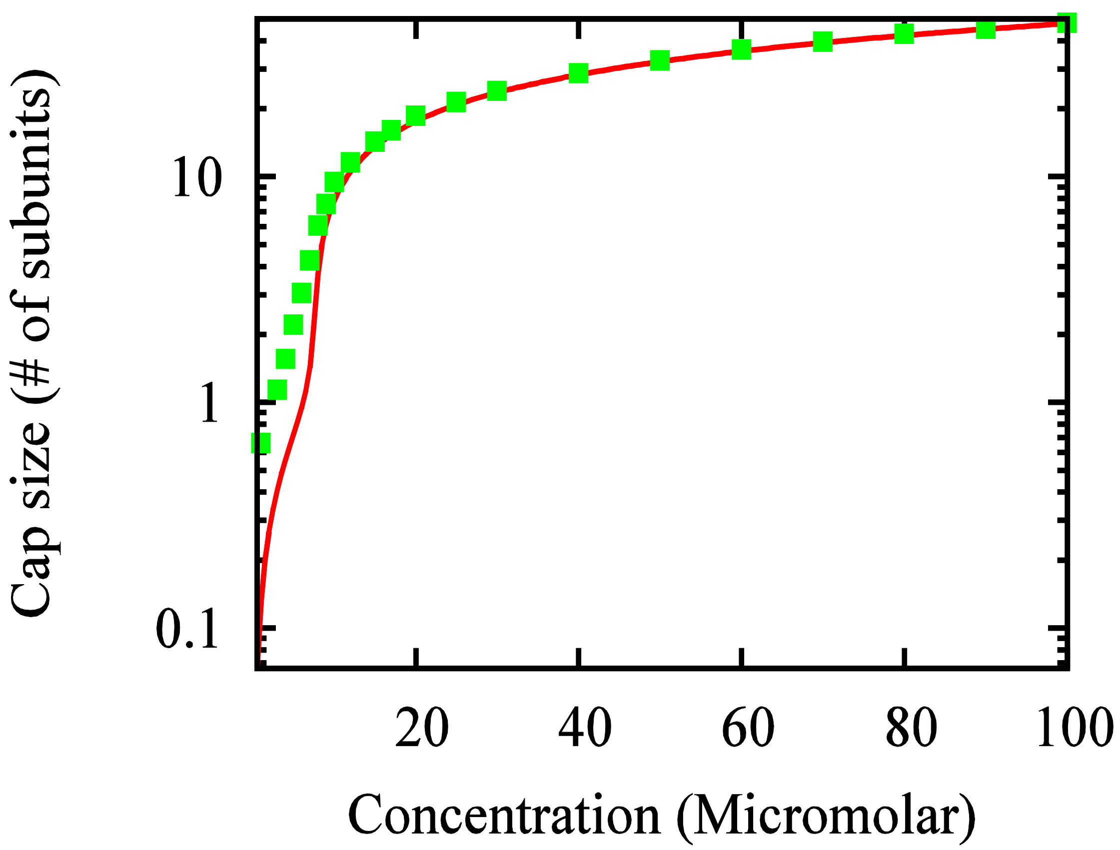
$f_r(N)$- rescue frequency = probability flux out of shrinking phase

We found N=2 adequately describes experimental data

Our Model

Average cap size as a function of tubulin concentration. Symbols – computer simulations, line – mean-field analytical solutions



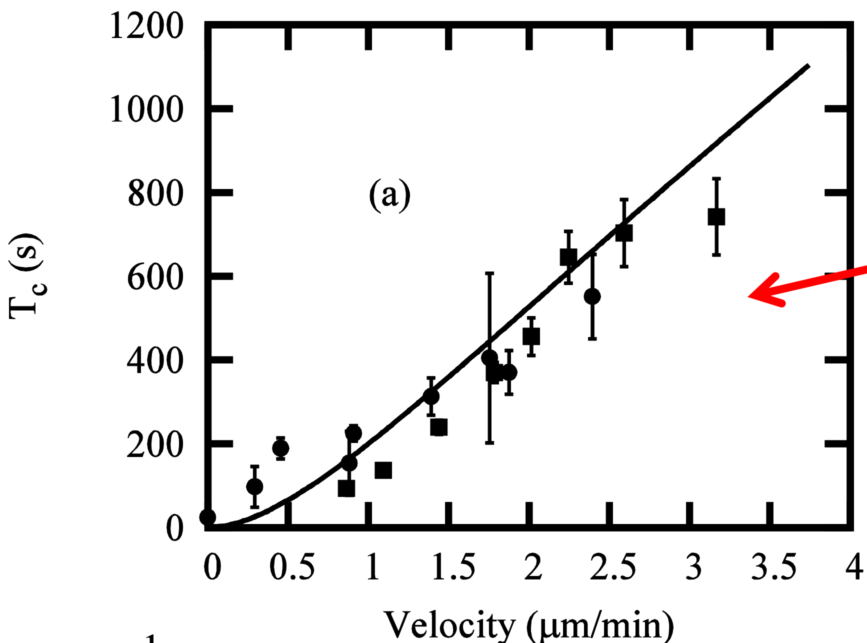


1) Critical concentration – 7 μM

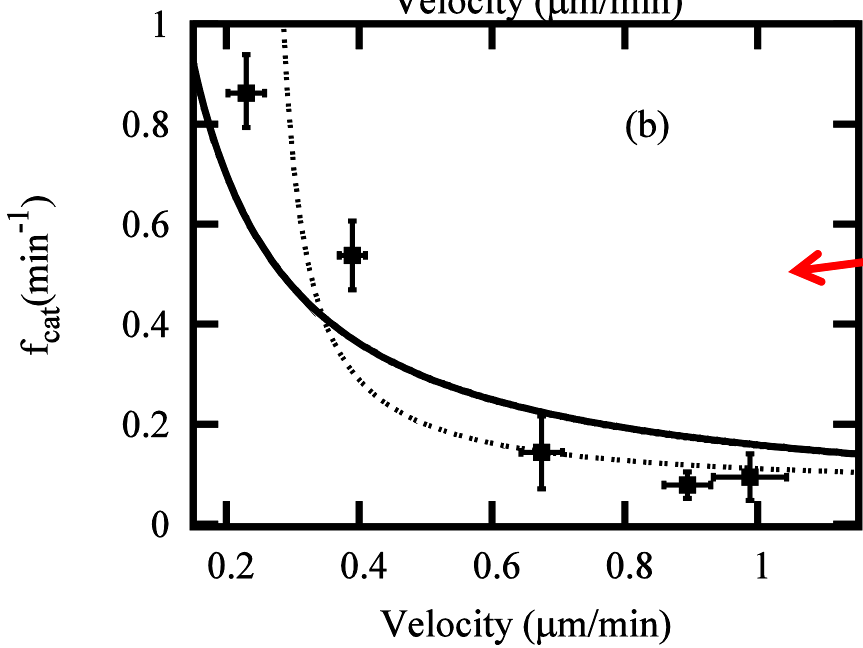
2) The cap size <50 subunits or 3.6 layers (28 nm), below optical resolution

3) Fluctuations are important below critical concentration

Our Model

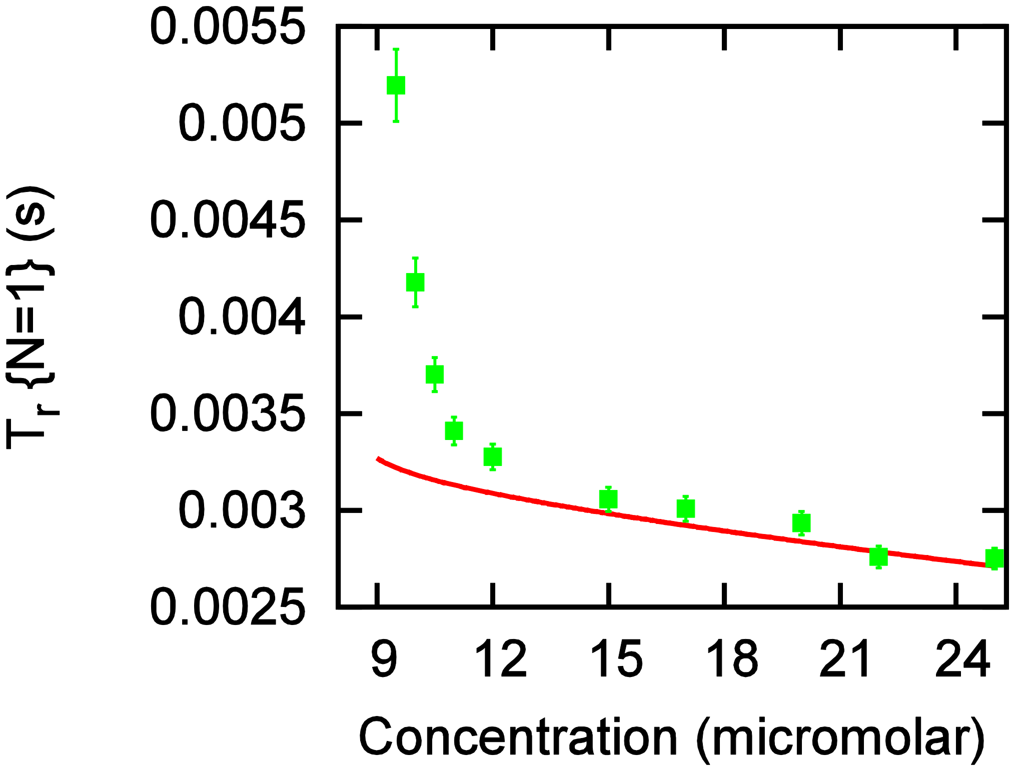


Time between catastrophes- comparison with experiments (circles) and simulations (squares)



Catastrophe frequency- comparison with experiments and phenomenological model (dotted curve)

Our Model



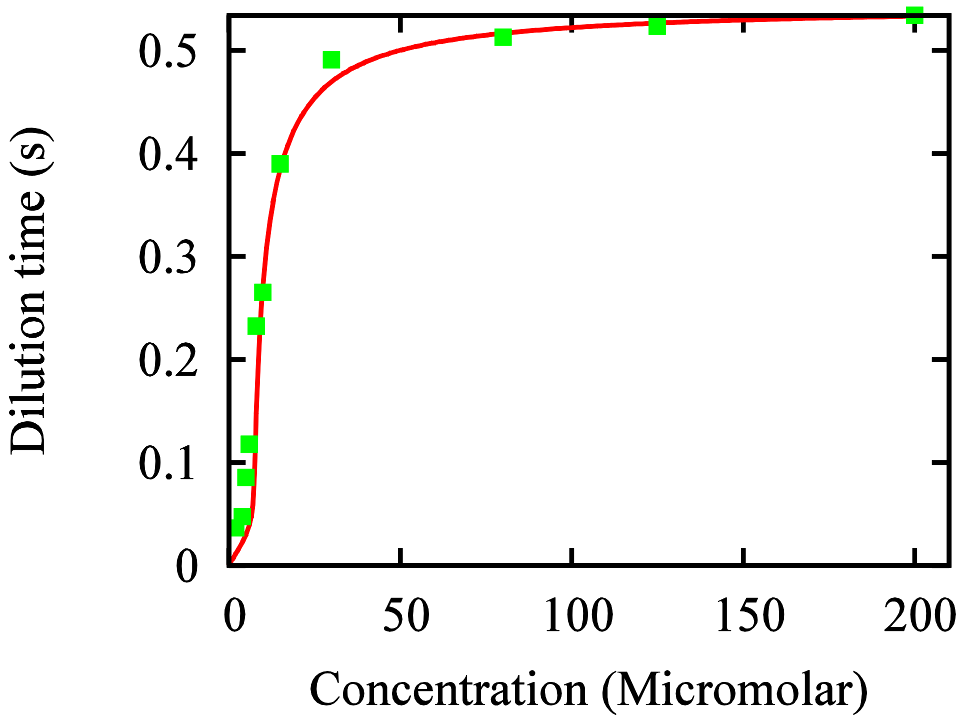
Rescue times as a function of tubulin concentrations as compared to simulations

Our model simultaneously accounts for catastrophes and rescues!!!

Our model predicts that rescues might be observable, but this is not the case in experiments. Why?

1) $T_r \sim 1/U$, but for short polymers they depolymerize before the rescue, $T_{collapse} \sim L/W_D$
2) At large U catastrophes are rare, so no rescues

Our Model



Delay times before catastrophes after dilution as a function of pre-dilution tubulin concentrations

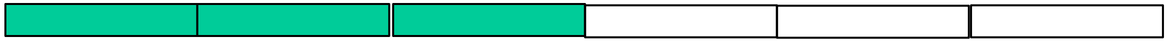
Experimental observations: 1) ~1s; 2) delay times are independent of concentrations above the critical concentration

PLoS ONE **4**, e6378 (2009)

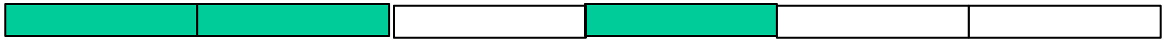
Role of Hydrolysis in Microtubule Dynamics

3 mechanisms of hydrolysis:

1) **Vectorial** – sharp front between hydrolyzed and unhydrolyzed subunits, hydrolysis only at the border with already hydrolyzed monomers



2) Random – hydrolysis can happen with equal probability at any subunit

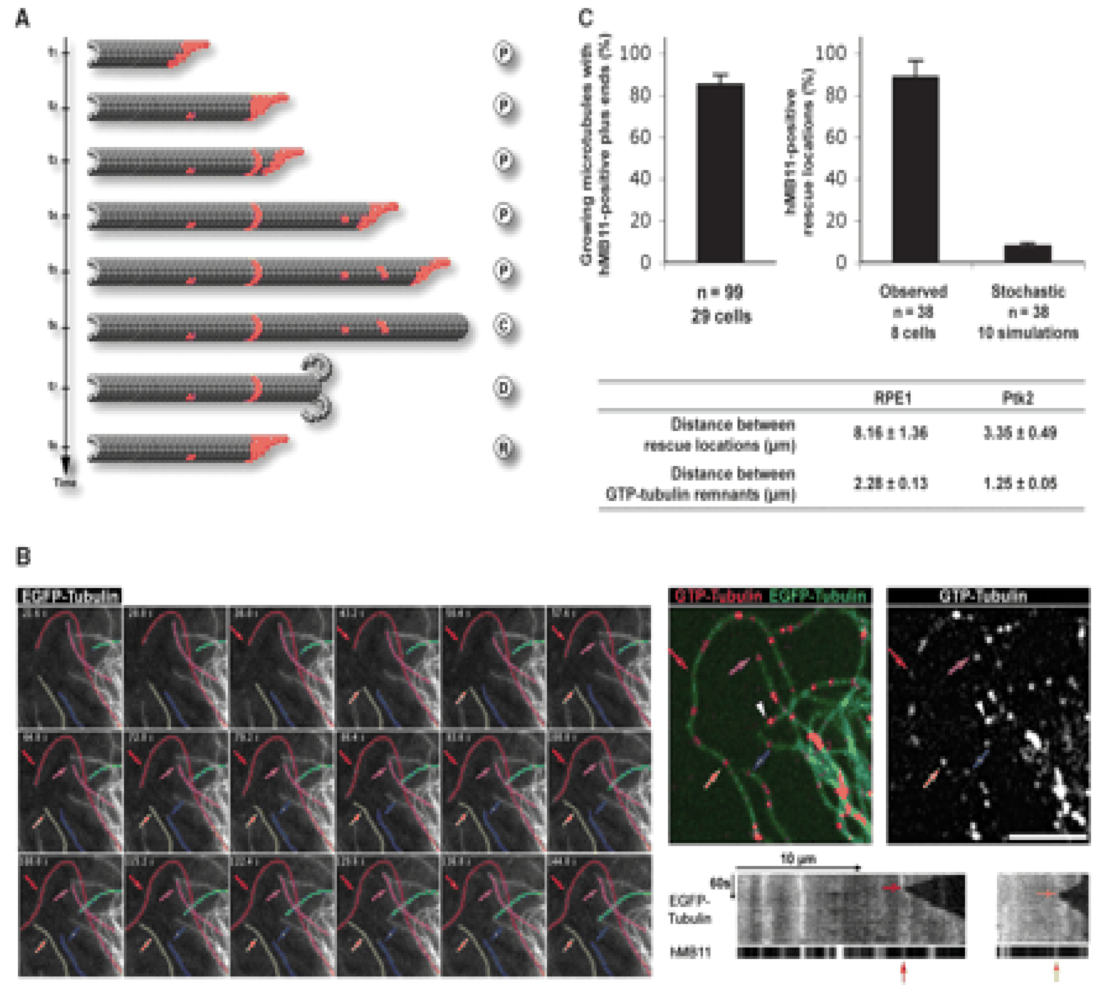


3) Cooperative – this mechanism interpolates between 2 limiting cases

Role of Hydrolysis in Microtubule Dynamics

mechanisms of hydrolysis:

It is still a controversial topic, but more recent experiments suggest that the mechanism is probably closer to the random or cooperative mechanisms

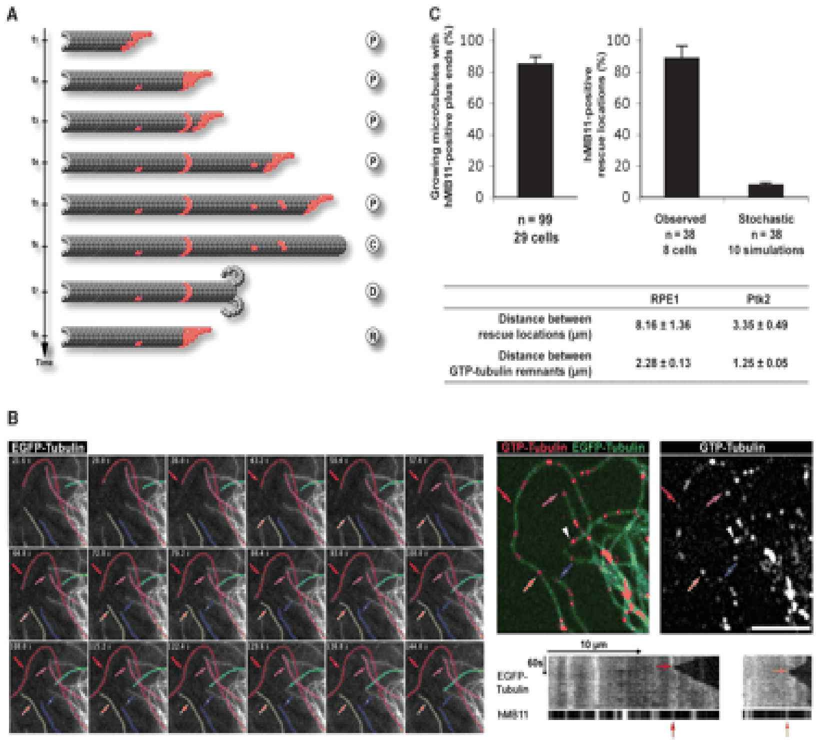


Science **322**, 1353 (2008)

Role of Hydrolysis in Microtubule Dynamics

Problems with current views of hydrolysis:

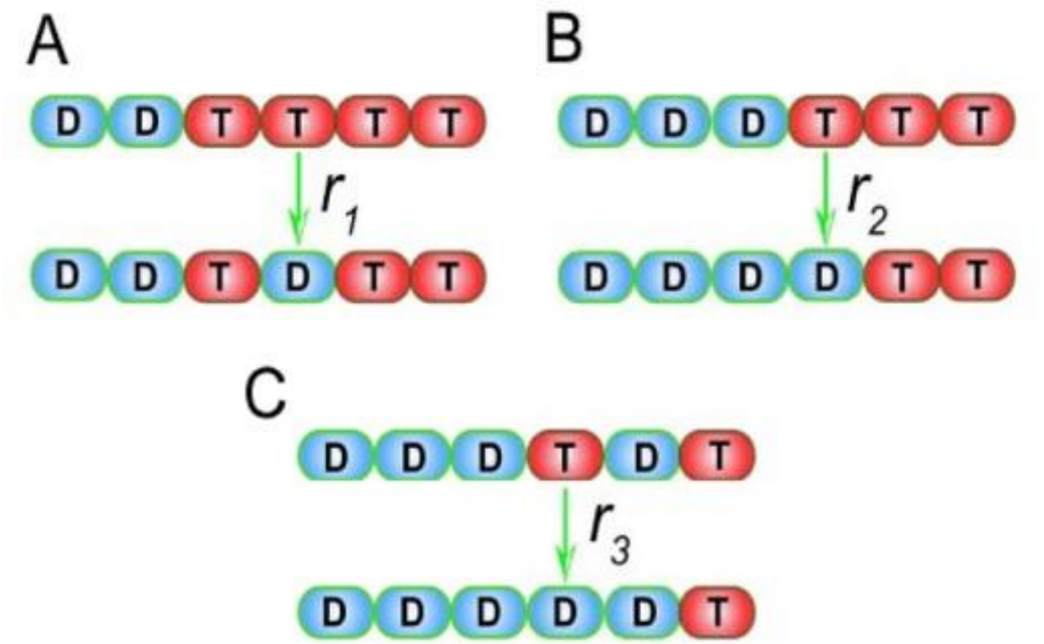
1) Phenomenological
2) Thermodynamically inconsistent
3) Neglect free-energy changes associated with corresponding biochemical processes



Role of Hydrolysis in Microtubule Dynamics

Our theoretical approach:

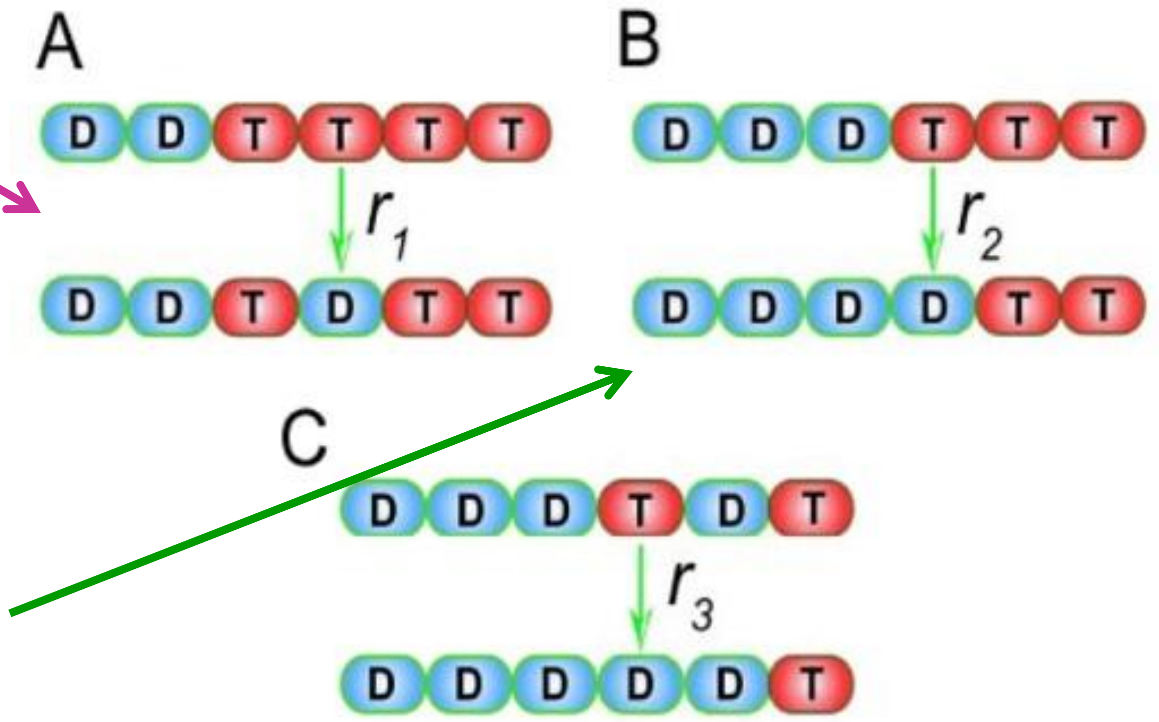
1) Microtubule is viewed as a single filament
2) Hydrolysis is a complex multi-state process, but we consider it as a 2-state process
3) Our main idea-thermodynamic: hydrolysis rates depend on free-energy differences before and after hydrolysis



T- unhydrolyzed
D-hydrolyzed

Role of Hydrolysis in Microtubule Dynamics

1) hydrolysis of the subunit surrounded by 2 T monomers



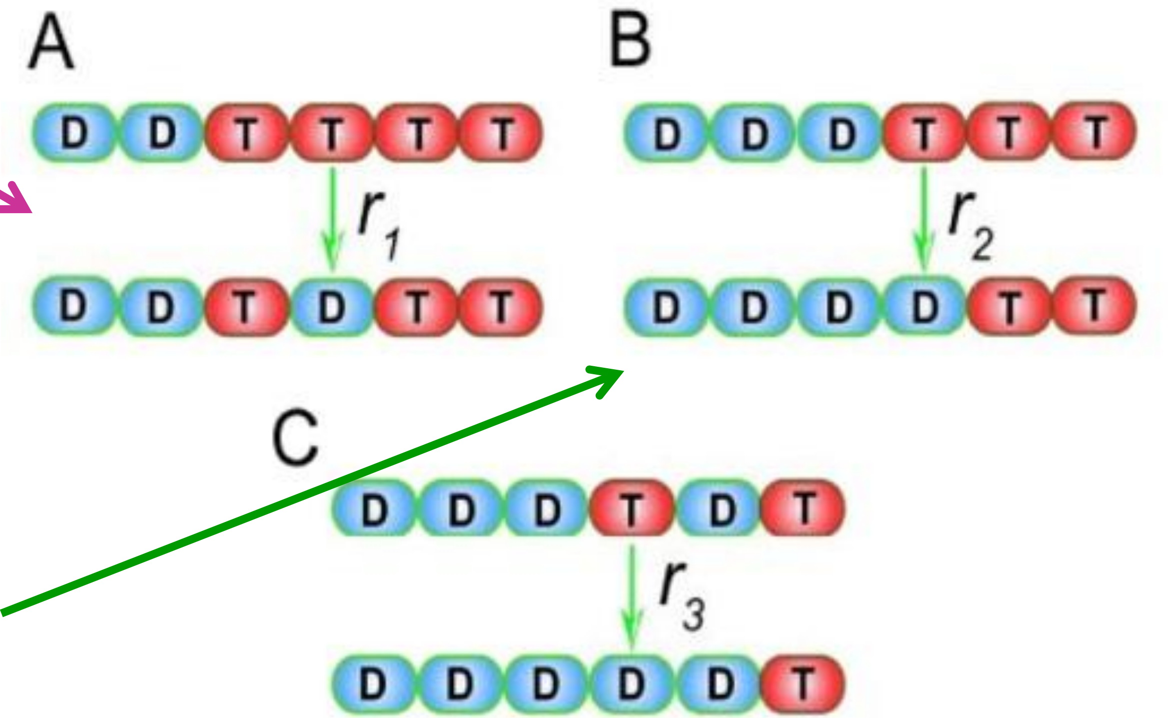
2) hydrolysis of the subunit surrounded by 1 T and 1 D monomers

3) hydrolysis of the subunit surrounded by 2 D monomers

Role of Hydrolysis in Microtubule Dynamics

1) hydrolysis of the subunit surrounded by 2 T monomers

$$\Delta G_1 = 2\varepsilon_{TD} - 2\varepsilon_{TT}$$



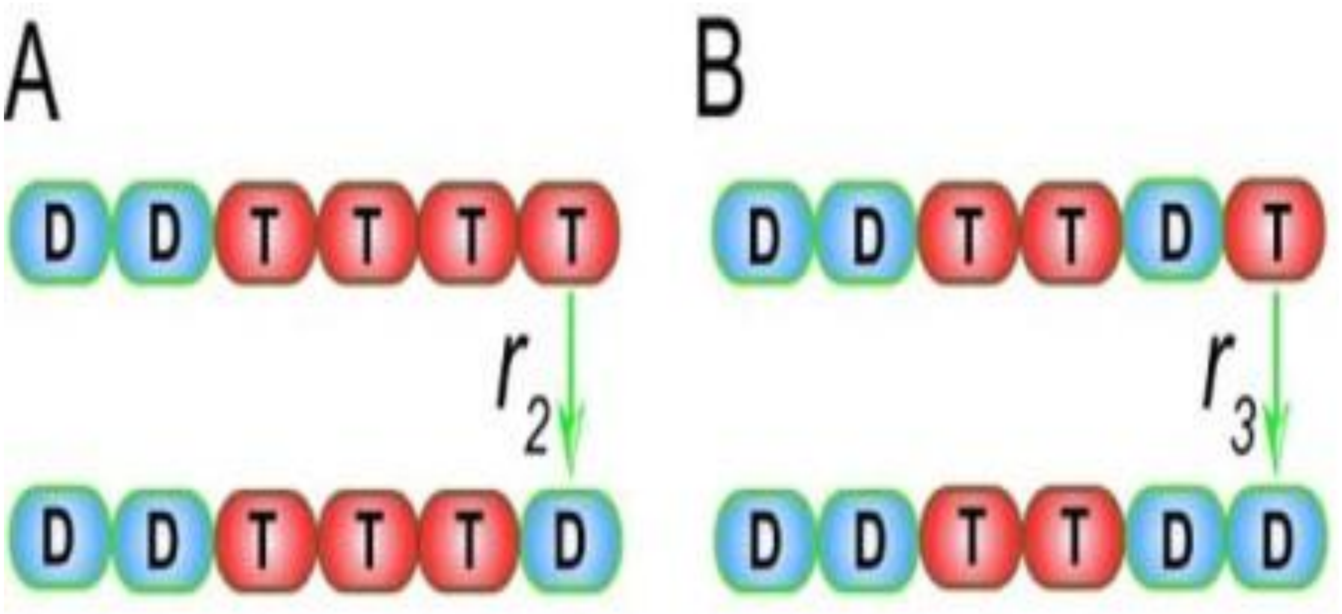
2) hydrolysis of the subunit surrounded by 1 T and 1 D monomers

$$\Delta G_2 = \varepsilon_{DD} - \varepsilon_{TT}$$

3) hydrolysis of the subunit surrounded by 2 D monomers

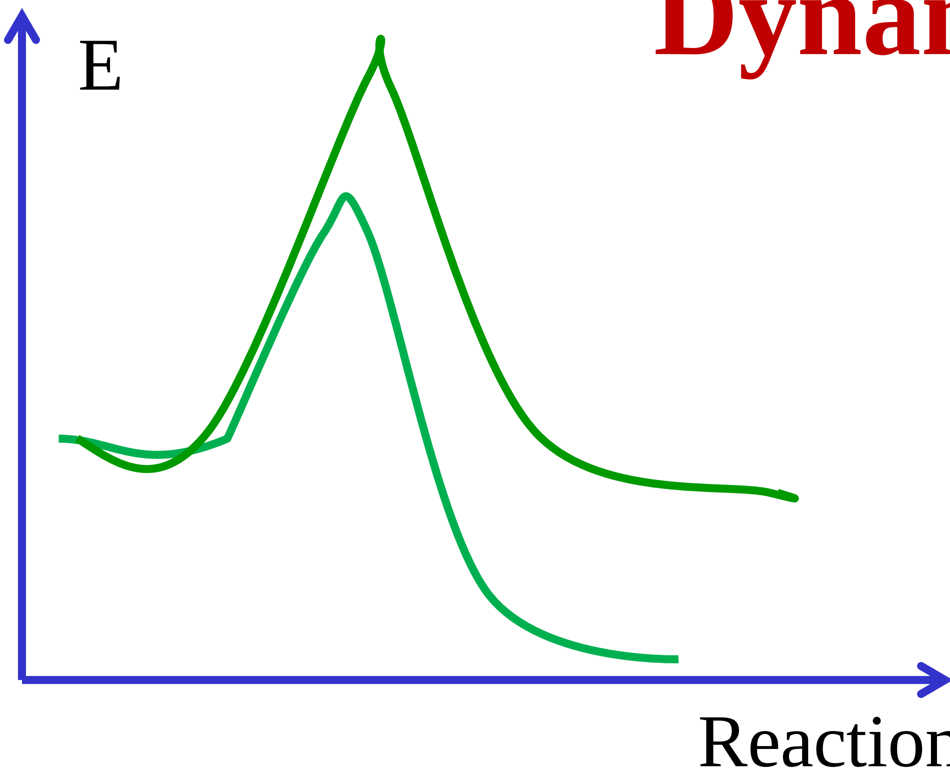
$$\Delta G_3 = 2\varepsilon_{DD} - 2\varepsilon_{TD}$$

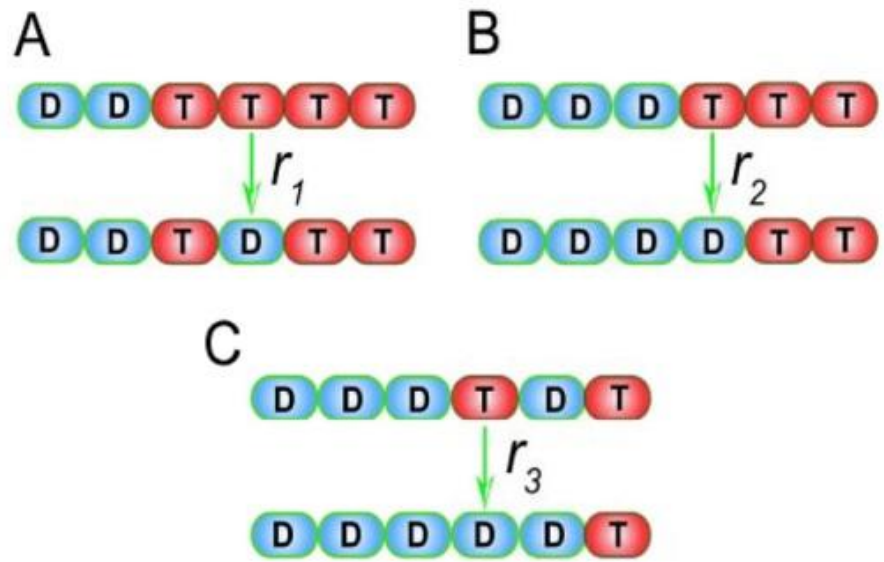
Role of Hydrolysis in Microtubule Dynamics



Hydrolysis processes at the end subunits also depend on chemical composition of configurations

Role of Hydrolysis in Microtubule Dynamics





$$\boldsymbol{r_i \cong exp(-\theta \Delta G_i / k_B \mathrm{T})}$$

0<θ<1 –relative distance to a transition state along the reaction coordinate

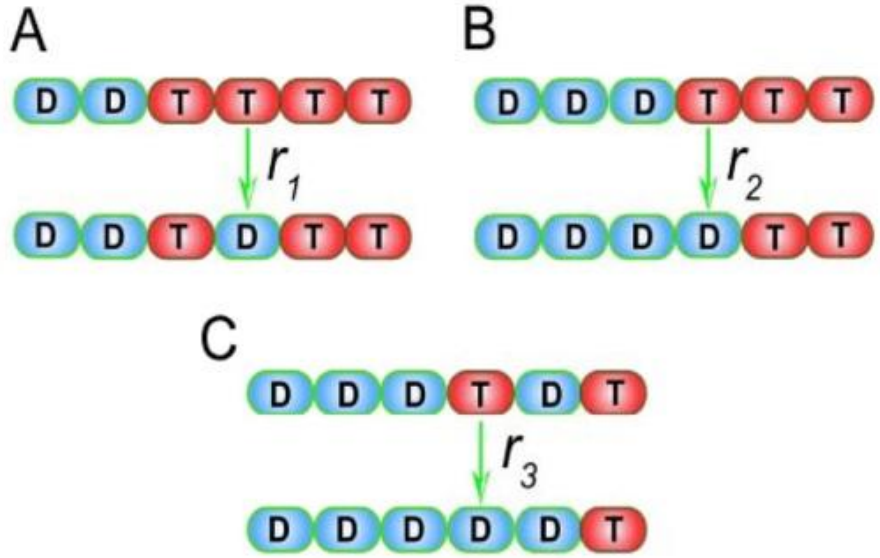
Role of Hydrolysis in Microtubule Dynamics

$$\varepsilon = 2\varepsilon_{TD} - \varepsilon_{TT} - \varepsilon_{DD}$$

relative cost of putting the hydrolyzed subunit in the filament

$$\Delta G_1 = 2\varepsilon + \Delta G_3$$

$$\Delta G_2 = \varepsilon + \Delta G_3$$



$$r_i \cong exp(-\theta \Delta G_i / k_B \mathrm{T})$$

$$\alpha = exp(-\theta \varepsilon / k_B \mathrm{T})$$

hydrolysis cooperativity parameter

Role of Hydrolysis in Microtubule Dynamics

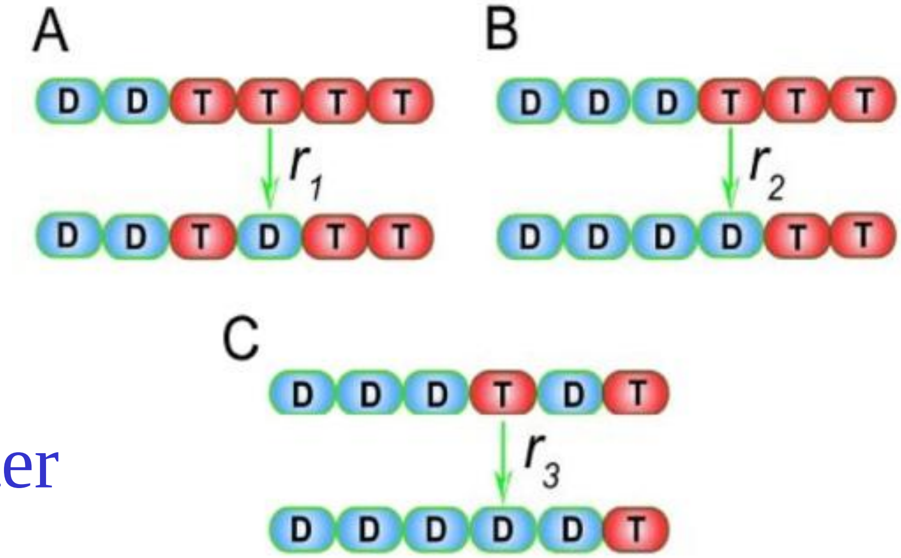
$$\varepsilon = 2\varepsilon_{TD} - \varepsilon_{TT} - \varepsilon_{DD}$$

$$\alpha = exp(-\theta\varepsilon/k_B \mathrm{T})$$

hydrolysis cooperativity parameter

For microtubules T-T have the strongest interactions

$$\varepsilon \approx -\varepsilon_{TT},\ 0 \leq \alpha \leq 1$$

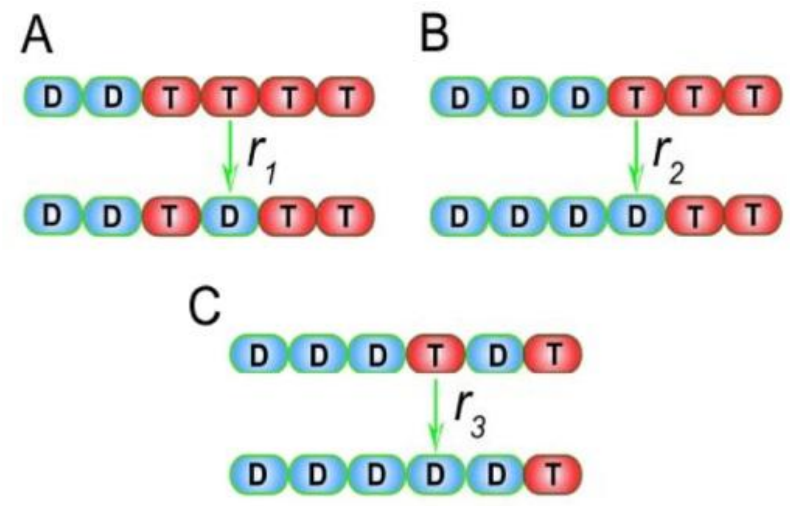


$$r_i \cong exp(-\theta\Delta G_i/k_B \mathrm{T})$$

Role of Hydrolysis in Microtubule Dynamics

$\varepsilon \approx -\varepsilon_{TT}$, $0 \leq \alpha \leq 1$

$\alpha = exp(-\theta\varepsilon/k_B T)$



Limiting behavior:

1) $\varepsilon=0$, $\alpha=1$ – weak cooperativity; random hydrolysis, the hydrolysis rate is independent of configuration
2) $\varepsilon \rightarrow \infty$, $\alpha=0$ – strong cooperativity; hydrolysis can only take place when both neighboring subunits are already hydrolyzed. Important: this is not a vectorial hydrolysis

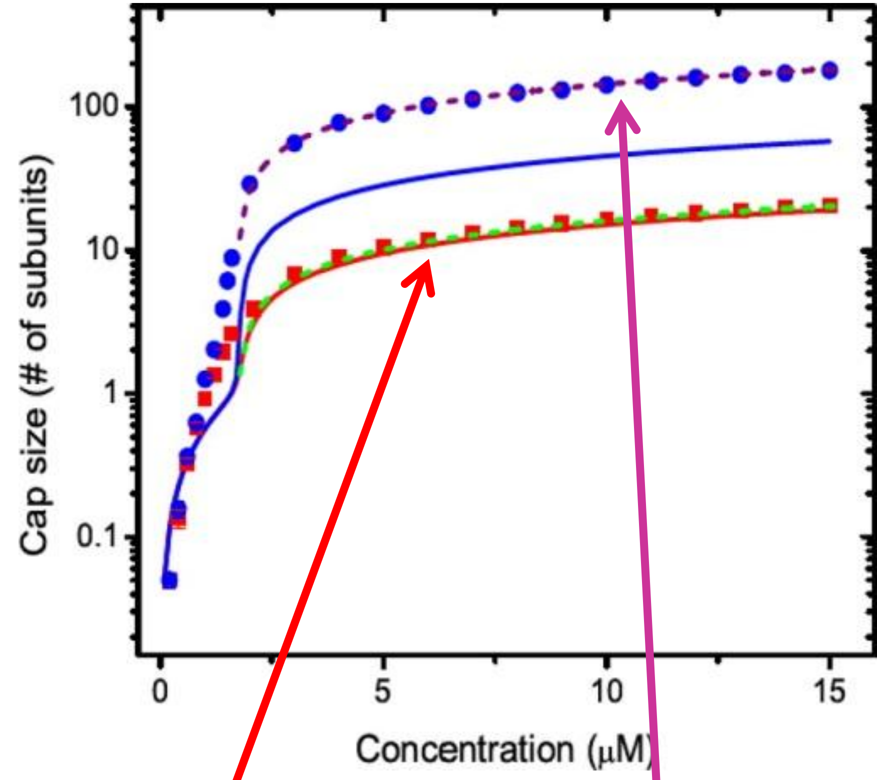
$r_i \cong exp(-\theta\Delta G_i/k_B T)$

$\Delta G_1 = 2\varepsilon + \Delta G_3$

$\Delta G_2 = \varepsilon + \Delta G_3$

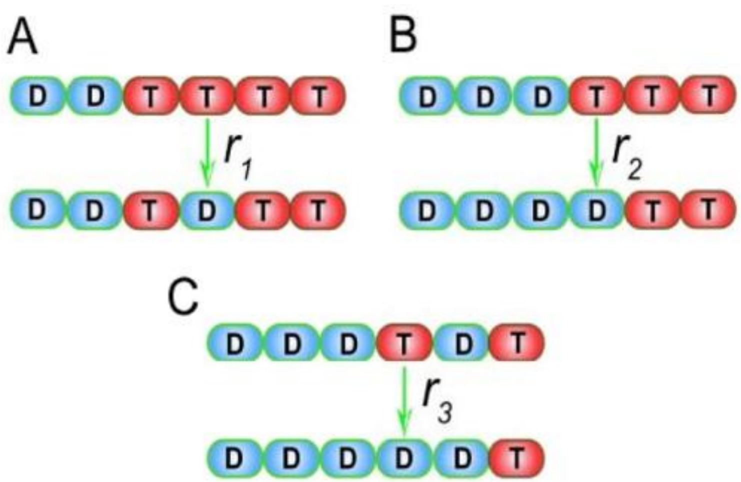
Role of Hydrolysis in Microtubule Dynamics

Increasing the cooperativity makes the length of unhydrolyzed cap larger above the critical concentration, because the rates for processes A and B are effectively smaller



weak cooperativity

strong cooperativity

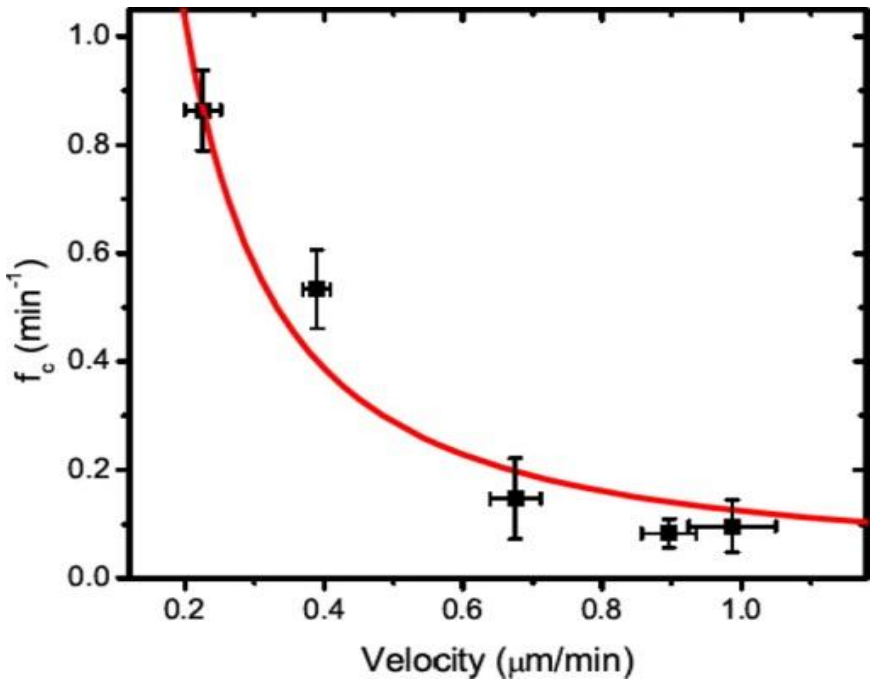


Role of Hydrolysis in Microtubule Dynamics

Our approach allows us to calculate exactly all dynamic properties of the filament.

Our analysis of expt. data suggests that microtubules can be described by a model with a weak cooperativity α=0.9 (almost random)

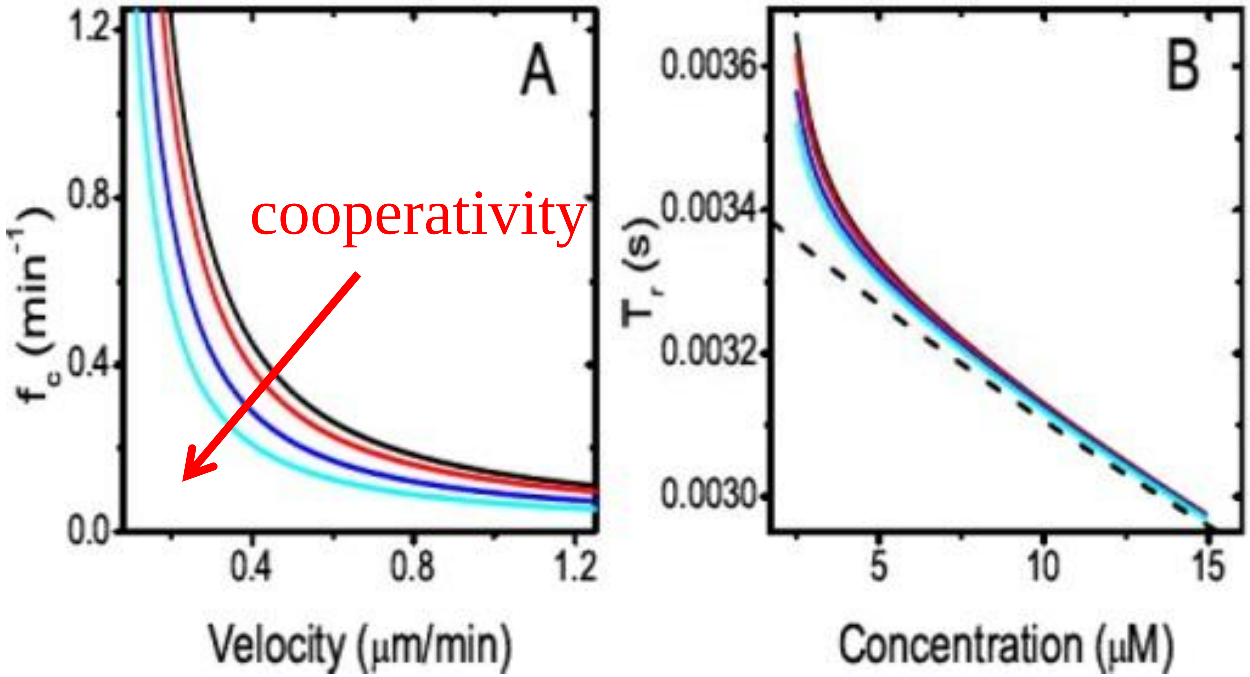
Frequency of catastrophes



Expt. Data from *Mol. Biol. Cell* 1992, **3**, 1141

Role of Hydrolysis in Microtubule Dynamics

Frequency of catastrophes | Time between rescues

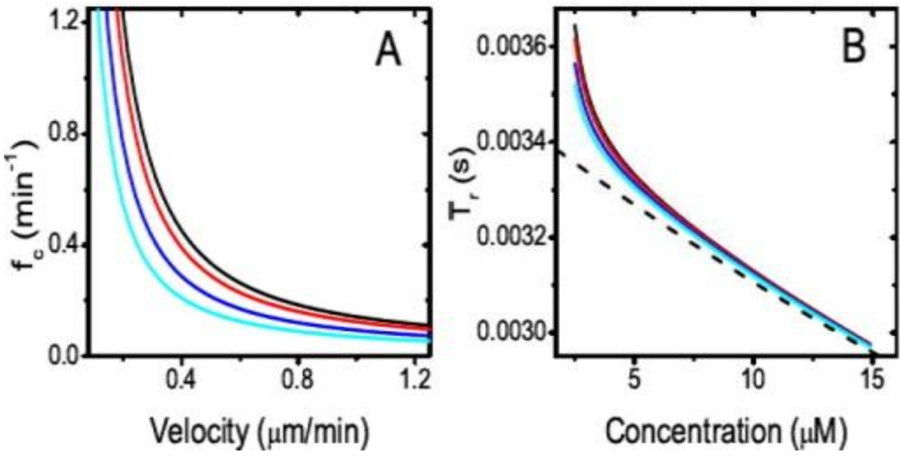


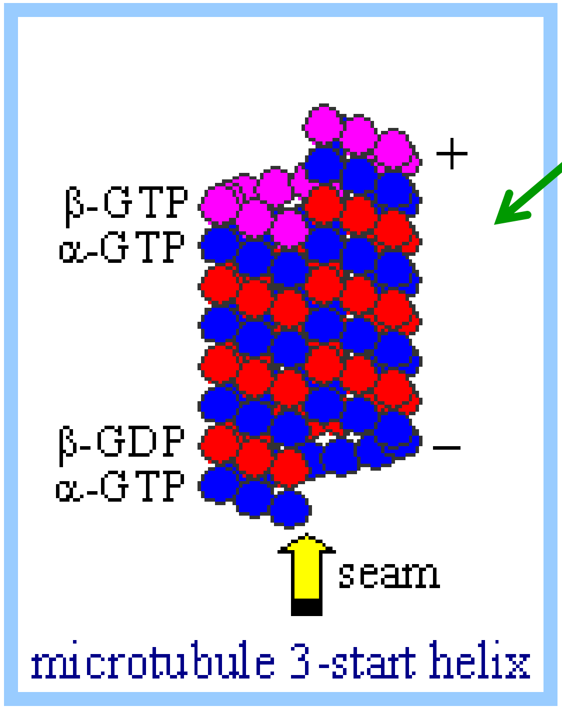
Increasing cooperativity lowers the frequency of catastrophes, but it does not have effect on rescues.

Why? Catastrophes depend on hydrolysis and dissociations, but rescues depend on association rates

Role of Hydrolysis in Microtubule Dynamics

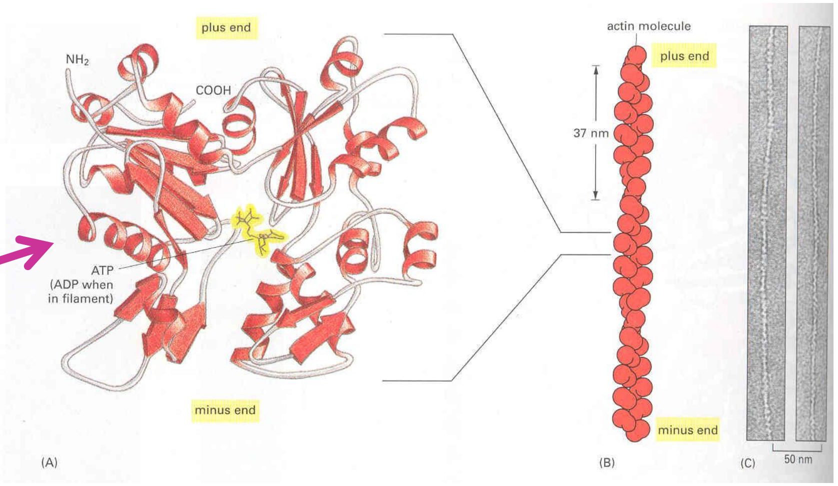
Question: why dynamic instability is observed in microtubules but not in actin filaments?





weak cooperativity

strong cooperativity



Critical View and Future Studies

- Our theoretical approach neglects mechanical degrees of freedom and their coupling to biochemical processes
- Mostly mean-field approaches, correlations are not taken into account
- Multi-filament structure for more complex models with hydrolysis is not accounted for
- The role actin-binding and microtubule binding proteins is not discussed
- Temporal changes in properties are not discussed, only steady-state calculations

Conclusions:

- **Analytical method of calculating dynamic properties of cytoskeleton proteins is developed**
- **Our approach is based on the discrete-state stochastic 1D nonequilibrium models that take into account relevant biochemical processes**
- **Our method simultaneously describes both rescues and catastrophes**
- **Allows us to understand the microscopic role of hydrolysis processes. Vectorial hydrolysis is unphysical**
- **It explains most experimental observations, supported by Monte Carlo simulations**

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Publications:

1) *Biophys. J.* 2012, **102**, 1274-1283
2) *J. Phys. Chem. B* 2013, **117**, 9217-9223